MORPHOLOGICAL AND ANATOMICAL RESPONSES TO PLANT GROWTH REGULATORS OF ABSCISED SHOOT APICES OF GRAPEVINES (VITIS) IN IN VITRO CULTURE AND HEAT INACTIVATION OF GRAPEVINE FANLEAF VIRUSES IN APICES

by

P G GOUSSARD

Dissertation presented for the Degree of
Doctor of Philosophy in Agriculture at
the University of Stellenbosch

FEBRUARY 1984 STELLENBOSCH

Dedicated to Laetitia,
Charl, Somari and Wehan

ACKNOWLEDGEMENTS

The author wishes to express his sincere gratitude and appreciation to the following persons and institutions:

Professor C.J. Orffer, promotor, for his guidance and valuable suggestions.

Professor J.A. de Bruyn, co-promotor, for his suggestions and editorial assistance.

Professor R.J. Weaver, external examiner, University of California.

Stellenbosch Farmers' Wineries for provision and maintenance of facilities for this project.

The Department of Agriculture for financial assistance.

The Director, Oenological and Viticultural Research Institute, Stellenbosch, for grapevine material and use of light microscope facilities.

The Director, Fruit and Fruit Technology Research Institute, Stellenbosch, for use of scanning electron microscope facilities.

The Director, Plant Protection Research Institute, Stellenbosch, for use of the Titertek Multiscan apparatus.

Mrs J. de V. Bezuidenhout and Mr A. van Rooyen for assistance with statistical planning and analysis.

Miss F. Jordaan for typing.

CONTENTS

SECTION 1

INTRODUCTION

Efficient vegetative procedures are essential for the successful propagation of many woody plant species. *In vitro* techniques have greatly increased the scope and potential of propagation by exploiting regenerative behaviour more efficiently and in a wider range of plants than is possible with conventional procedures.

Shoot apex culture to produce dahlias free of dahlia mosaic virus was first described by Morel and Martin (1952). In 1960 Morel demonstrated with orchids that this technique enabled clonal multiplication rates that were several times faster than conventional methods. Ever since this pioneering work, *in vitro* techniques have become widely adopted in plant propagation with principle applications such as: (a) production and maintenance of pathogen-free plants and (b) rapid aseptic multiplication under controlled conditions throughout the year and without any seasonal effects.

Growth substances play a major role in controlling growth responses of shoot apices cultured *in vitro*. The finding (Skoog and Tsui, 1948; Skoog and Miller, 1957) that high auxin/cytokinin balances favoured callusing and rooting in tobacco callus cultures whereas shoot initiation occurred with high levels of cytokinin/auxin, opened the field for *in vitro* propagation of many plants. Media supplying high concentrations of cytokinins

and little or no auxin are used to enhance shoot production from excised shoot apices (Murashige, 1977; Hussey, 1980; Wang and Hu, 1980; Allan, 1981a,b). Shoots produced with rapid multiplication techniques may be cultured similarly or transferred to an auxin-containing medium to grow to complete plants (Murashige, 1977; Abbott, 1978; Hussey, 1980).

Shoot apices of herbaceous plants are widely used for in vitro propagation, but those of woody plant species to a lesser degree (Murashige, 1974, 1977; Abbott, 1978; Anderson, 1980). Significant progress has recently been made in the development of in vitro techniques for successful propagation of woody species (Cheng, 1978). In grapevines many researchers (Gifford and Hewitt, 1961; Galzy, 1964, 1972; Hoeffer and Gifford, 1964; Wolfswinkel, 1965; Bini, 1976) attempted to produce plantlets from excised shoot apices. Considerable difficulties were encountered and recovery percentages were very low.

In vitro mass propagation of grapevines was first reported by Barlass and Skene (1978, 1979). They succeeded in obtaining adventitious shoot production from fragmented shoot apices followed by subsequent clonal multiplication. Apart from brief references by Jako and Nitsch (1980) and Chée and Pool (1982), knowledge is inadequate of optimal growth substance requirements for the successful in vitro culturing of non-fragmented shoot apices.

Various authors have confirmed that practical methods of inactivating viruses from vegetatively propagated species include

thermotherapy of infected parent plants, or shoot apex culture, or a combination of both. Success in obtaining plants free of particular viruses may depend upon the initial size of the shoot apex excised, as reported by Stone (1968) with carnations infected with carnation mottle, vein mottle and latent viruses. Most workers use excisions measuring 0,5 - 1 mm, consisting of the apical dome plus 2 – 3 leaf primordia. It has, however, been clearly indicated that the probability of recovering healthy plants from infected stock is greater with smaller shoot apex explants (Limasett and Cornuet, 1949; Stone, 1963; Mori and Hosokawa, 1977).

Heat therapy (38°C) over prolonged periods to inactivate grapevine viruses often result in suppressed root and shoot growth coupled with leaf abnormalities such as chlorosis and various deformations (Kriedemann et al., 1976; Brückbauer, 1979). Mist propagating of shoot tips (1 - 2 cm) taken from potted heat-treated vines often gave low percentages of rooting and recovery (Bovey, 1980). Successful culturing of small excisions of shoot apices, alone or in combination with heat therapy, therefore, offers the possibility of increased rates of virus inactivation.

Present knowledge regarding successful in vitro culturing of small apices and apical meristems of shoots of grapevine remains fragmentary. The main objectives of this study were to investigate: (1) morphological and anatomical responses of shoot apices to several growth substances included in culture media, (2) effect of size of excised shoot apices on successful

culturing and (3) inactivation of grapevine fanleaf virus disease (GFLV) by heat treating apical regions of rapidly elongating shoots of potted vines only as compared to small detached segments of shoots (0,5 mm) without roots grown *in vitro*.

SECTION 2

LITERATURE

2.1 Morphological responses of shoot apices of herbaceous and woody plant species cultured in vitro under varying conditions

2.1.1 Culture media

2.1.1.1 Inorganic components

A number of standard salt mixtures is outlined by Gamborg et al. (1976). The inorganic fraction of nutrient media usually consists of either the media of White (1943), or of the basic (MS-62) or high salt (revised) (RM-62) media of Murashige and Skoog (1962), or modifications thereof. Minor elements recommended by Berthelot (1934) or Heller (1953) have been generally incorporated into media. Iron has been supplied in various forms such as $FeSO_4$, $FeC\ell_3 \cdot 6H_2O$ (White, 1963), or chelated iron (Murashige and Skoog, 1962; Wilmar et al., 1964).

Shoot apices of carnation have been successfully cultured on Knop's (1865) macro-nutrient solution (Quak, 1957; Baker and Phillips, 1962; Stone, 1963; Van Os, 1964); White's (1954) medium (Hackett and Anderson, 1967) and RM-62 (Izvorska and Rangelova, 1974). In strawberries, Knop's (1865), White's (1943) and RM-62 media have been successfully employed (Belkengren and Miller, 1962; Miller and Belkengren, 1963; McGrew, 1965; Adams, 1972; Earle and Langhans, 1975; Boxus, 1976). Earle and Langhans (1974) used RM-62 in in vitro propagation of chrysanthenum.

The RM-62 medium has been widely employed for successful culturing shoot apices of woody plant species, e.g. almond-peach hybrids (Tabachnik and Kester, 1977); apples (Abbott and Whiteley, 1976; Jones et al., 1977); blackberries (Broome and Zimmerman, 1978); bougainvillea (Chaturvedi et al., 1978); gooseberries (Jones and Vine, 1968); prunus (Boxus, 1975; Quoirin and Lepoivre, 1977); Rosa multiflora (Elliot, 1970); rhododendrons (Anderson, 1975) and stone fruits (Jacob, 1974).

In grapevines, researchers used Knop's (1865) macro-nutrient medium in attempts to produce plantlets from excised shoot apices in vitro (Gifford and Hewitt, 1961; Hoeffer and Gifford, 1964; Wolfswinkel, 1965; Galzy, 1972; Bini, 1976; Grenan, 1979). More recently macro- and micro-nutrient levels used by Murashige and Skoog (1962) have been successfully applied (Jako and Nitsch, 1980; Chée and Pool, 1982; Harris and Stevenson, 1982). Barlass and Skene (1978, 1979) successfully cultured fragmented shoot apices on MS-62.

2.1.1.2 Sugar

Sugars are used either in the form of sucrose or glucose or both. Brants (1968) reported that sucrose gave much better growth of Freesia than glucose. Glucose at 20 g/ℓ resulted in poor rooting of carnations, whilst the process was stimulated at 40 g/ℓ (Phillips, 1968). Explants of the hybrid tea rose cv. Superstar responded well to both sucrose and glucose, each at 10 g/ℓ (Jacobs et al., 1969).

2.1.1.3 Vitamins and organic substances

One or more vitamins are frequently utilised. In general, the vitamins recommended by Murashige and Skoog (1962) are widely used. For carnations addition of calcium panthothenate, inositol, biotin, niacin, pyridoxine and thiamine supported the growth of detached shoot apices (Stone, 1963). In grapevines, Galzy (1964) and Wolfswinkel (1965) included biotin, calcium panthothenate, casein hydrolysate, cysteine, inositol, niacin, pyridoxine and thiamine. Undefined organic supplements such as coconut milk and yeast extract showed no stimulatory effects on rooting (Wolfswinkel, 1965).

2.1.1.4 Growth substances

Skoog and Tsui (1948) and Skoog and Miller (1957) established that organogenesis in cultured plant tissues could be manipulated through growth substances. Promotive effects of auxins on root initiation and of cytokinins on shoot initiation have been well illustrated with tissue cultures (Haissig, 1965; Winton, 1968; Wolter, 1968; Abo El-Nil and Hildebrandt, 1971; Grinblat, 1972; Yie and Liaw, 1977).

As regards carnations, Stone (1963) stated that one of the major factors affecting rooting of excised shoot apices is the presence of exogenous auxin. Evidence for promotive effects of 1-naphthaleneacetic acid (NAA) on rooting is offered by Stone (1963); Van Os (1964); Jacobs *et al*. (1969); Hollings and Stone (1970); Jacob (1974) and Earle and Langhans (1975). In *Nicotiana rustica* (Walkey *et al*., 1969); tea rose cv. Superstar (Jacobs, 1973); stone fruits (Jacob, 1974) and *Humulus lupulus* (Adams, 1975),

rooting was favoured by indole-3-acetic acid (IAA). Indole-3-butyric acid (IBA) promoted rooting of gooseberries (Jones and Vine, 1968); strawberries (Boxus, 1974) and Prunus species (Boxus, 1975). In some cases promotive effects of 2,4-dichlorophenoxyacetic acid (2,4-D) on rooting were reported (Jacobs, 1973; Jacob, 1974; Adams, 1975; Earle and Langhans, 1975).

In orchids, gibberellic acid (GA_3) was essential in tissue organisation, suppressing callus initiation and enabling the shoot apex to differentiate a stem instead of an undifferentiated mass of callus (Morel, 1964a,b). Carnation shoot apices are mostly cultured without GA_3 (Quak, 1957; Baker and Phillips, 1962; Stone, 1963; Phillips and Matthews, 1964). No beneficial effects of GA_3 on growth of rose Superstar shoot apices were reported (Jacobs, 1970a,b). In the majority of cases marked elongation of explants occurred in the presence of GA_3 (Jones and Vine, 1968; Vine and Jones, 1969; Earle and Langhans, 1975). As GA_3 suppresses root initiation for carnations (Quak, 1977), explants are usually cultured without GA_3. Rooting in the presence of GA_3 was, however, reported by Jacob (1974) with stone fruits and by Pennazio (1975) with carnations.

Promotive effects of cytokinins were reported by Jacobs, 1973; Boxus, 1974; Jacob, 1974; Adams, 1975; Abbott and Whiteley, 1976; Ma and Wang, 1977; Mekers, 1977 and Preil and Engelhardt, 1977. Kinetin promoted growth of carnation and rose shoot apices although rooting was suppressed (Phillips, 1968; Jacobs et al., 1969). Stimulatory effects of 6-benzylaminopurine (BAP) were reported by Jones (1967) and Adams (1975) for explant growth in

apples and lupins respectively. Detached shoot apices of roses were dependent on the presence of either kinetin, BAP or zeatin to respond with shoot elongation and leaf expansion (Elliot, 1970; Jacobs, 1973). Suppressive effects of cytokinins on rooting were confirmed by several authors (Jones, 1967; Phillips, 1968; Jacobs et al., 1969; Boxus, 1974).

Multiple shoot production from detached shoot apices in vitro can possibly be explained in that cytokinins may effect a release of axillary meristems from apical dominance (Wickson and Thimann, 1958). Shoot apices of herbaceous plants are widely used for clonal multiplication in vitro, but those of woody species to a lesser degree (Murashige, 1974, 1977; Sagawa, 1976; Abbott, 1978; Cheng, 1978; Hussey, 1980). In this multiplication process, masses of proliferating shoots are produced, arising adventitiously or as axillary growths when apical dominance is suppressed (Murashige, 1977; Abbott, 1978; Hussey, 1980). High concentrations of cytokinins and little or no auxin (Murashige, 1977; Hussey, 1980) are used to enhance axillary branching.

Ma and Wang (1977) reported kinetin to be essential for the production of axillary shoots in Azalea. In papaya multiple shoots were produced with BAP (Yie and Liaw, 1977) and in Lilium with BAP in combination with low concentrations of NAA (Hussey, 1977). Detached shoot apices of woody plant species (apple, cherry, pear and plum) responded with axillary shoot production to combined applications of BAP and NAA (Cheng, 1978). Enhanced shoot production resulted in almond-peach hybrids (Tabachnik and

Kester, 1977); apples (Abbott and Whiteley, 1976; Jones et al., 1977); blackberries (Broome and Zimmerman, 1978) and bougainvillea (Chaturvedi et al., 1978) where culture media had been enriched with BAP. In grapevines, combinations of BAP and NAA were used (Jako and Nitsch, 1980; Chée and Pool, 1982). Although limited shoot numbers resulted, the potential of cytokinin:auxin combinations for clonal multiplication was indicated (Chée and Pool, 1982). In culturing fragmented shoot apices, the production of adventitious shoots was stimulated by BAP (Barlass and Skene, 1978, 1979).

Vollmer (1976) ascertained that cytokinin levels in tips of actively growing grapevine shoots exceeded those of the young subapical leaves. The question arises as to whether cytokinins remain present in apices once excised and whether small excisions will have the potential to produce cytokinins when cultured in vitro. Pool (1975) stated that zeatin riboside (ZR) is the main cytokinin in xylem exudate. He also reported that ZR favoured the in vitro development of ovaries and filaments of excised Concord grape flowers (prior to bloom). Budburst and shoot elongation of Concord cultured in vitro from non-flowering unrooted pieces of shoots increased dramatically in response to ZR treatments (Pool, 1974). References are lacking on responses of in vitro cultured non-fragmented shoot apices to cytokinins other than BAP.

Although excised shoot apices can be grown to intact plants without transferring to a different medium, in the majority of cases

it has been found necessary or beneficial to grow explants initially on a medium that induces either shoot or root growth and then to transfer them to a different medium to induce the counterpart (Jones and Vine, 1968; Vine, 1968; Adams, 1972; Cohen, 1977; Murashige, 1977; Theiler, 1977; Hussey, 1980; Wang and Hu, 1980). One method of carnation shoot apex culture involved the transfer of explants, once rooted, from a medium containing auxin to an auxin-free medium (Stone, 1963). This method implies that exogenous auxin is essential for rooting but is less important or even harmful for root elongation. Nishizawa and Nishi (1966) kept the sprouts of lily plants in a NAA solution prior to excision of the apex, thus benefiting the explant by the temporary presence of the growth substance and avoiding later toxicity.

In grapevines, previous researchers (Gifford and Hewitt, 1961; Galzy, 1972; Bini, 1976) attempted to stimulate rooting of excised shoot apices with an auxin treatment and then to induce shoot growth by lowering the auxin concentration. Methods of inducing the proliferation of shoots which are then rooted, appeared much more effective (Barlass and Skene, 1978, 1979; Jako and Nitsch, 1980; Chée and Pool, 1982).

In mass propagation techniques serial transferring of shoot clusters to cytokinin-containing media are used to enhance shoot production (Murashige, 1974, 1977; Abbott, 1978; Hussey, 1980; Wang and Hu, 1980). Rooting of excised shoots usually occurs on transfer to media lacking cytokinin but containing auxin

(Murashige, 1977; Hussey, 1978, 1980). Barlass and Skene (1978, 1979) achieved successful rooting of grapevine shoot tips on a medium lacking auxin, whereas the necessity of NAA was reported by Chée and Pool (1982).

2.1.1.5 The pH of nutrient media

The pH of a medium is usually adjusted to the range of 5 to 6 (Murashige, 1966). The RM-62 medium is widely used at pH 5,7 - 5,8. Carnation shoot apices grew considerably better at pH 5,5 than at pH 6 (Stone, 1963).

2.1.2 Light and temperature requirements

The environmental conditions for successive culturing excised shoot apices vary. Even when fully provided nutritionally, many tissue cultures were unable to respond due to unfavourable illumination and/or temperature regimes (Murashige, 1977). Stone (1963), Van Os (1964) and Alphi (1965) stated that low light intensities (1 000 - 3 000 lux) are widely used in mass propagation procedures. Final root initiation steps may, however, require higher intensities (3 000 - 10 000 lux) (Murashige, 1977). A 16h illumination period is widely used although a few reports suggested that daylength may be critical for shoot production in certain species (Murashige, 1977; Hussey, 1980). No differences were reported with an illumination period varying from 4 to 20 hours in culturing detached shoot apices of rhubarb (Hasegawa et al., 1973). In Pelargonium hortorum a definite dark period was found necessary to minimize the inhibitory effects of polyphenolic substances (Pillai and Hildebrandt, 1968).

In general, tissue cultures are most frequently incubated within the range of 25 - 27°C (Murashige, 1977). Hussey (1980) suggested that although most cultures will respond within the range of 15 - 25°C, normal environmental requirements of each species should be taken into account when deciding in the particular temperature to be used.

2.2 Morphology/anatomy of shoot apices and supplementary tissue of vascular plants

2.2.1 Structure

The structure of shoot apices in vascular plants has been reviewed by Foster, 1941; Philipson, 1949; Gifford, 1950, 1954; Popham, 1951; Rouffa and Gunckel, 1951; Buvat, 1952; Vaughan, 1952; Clowes, 1961 and Romberg, 1963. The tunica-corpus concept (Schmidt, 1924) of structure and growth of shoot apices for the angiosperms is well outlined (Foster, 1939; Ball, 1941; Reeve, 1948; Gifford, 1950, 1954; Philipson, 1954; Buvat, 1955; Sunderland, 1961; Romberg, 1963). The apical meristem refers to the smooth-surfaced apical dome alone and is composed of well-pronounced meristematic cells. Definite zones, having specialized functions, are found in the shoot apex. The tunica, composed of several layers, forms the peripheral zone with an anticlinal cellular arrangement. Beneath the tunica is the central mother-cell group which is the main part of the corpus and from which originates the pith rib meristem. Foliar initials originate as a result of mitotic activity of an initiating ring segment - produced by the peripheral zone - inducing leaf buttresses by division of dense meristematic cells. Enlargement

of shoot apices, which results from a cyclic growing movement, with subsequent initiation of leaf primordia and axillary buds, is referred to by Garrison, 1949; Gifford, 1950, 1954; Rouffa and Gunckel, 1951; Buvat, 1952, 1955 and Clowes, 1961.

In the grapevine, the apical meristem of the shoot has been regarded as consisting of one or two tunica layers over a poorly defined corpus (Branas, 1957; Hegedüs, 1957; Pratt, 1959, 1974; Thompson and Olmo, 1963). Leaf primordia are initiated on the flank of the apical meristem by periclinal divisions in the second layer. Subsequent divisions in various planes lead to the formation of a distinct protuberance (Pratt, 1959, 1974). Tendril primordia are initiated by periclinal divisions in the third layer of the flank of the apical meristem (Tucker and Hoefert, 1968). Except for V. labrusca (with a tendril opposite the leaf at nearly every upper node), the cultivated species of Vitis follow the discontinuous pattern of tendrils of V. vinifera (Viala and Péchoutre, 1910; Kroemer, 1923).

Axillary meristems originate somewhat later than the leaves subtending them (Seeliger, 1954; Sussex, 1955). In grapevines the latter was illustrated by Pratt (1959, 1974) in shoot apices of V. labrusca.

2.2.2 Size

Comparative studies of dormant and growing shoot apices, with detailed measurements of apical meristems, were carried out by Rouffa and Gunckel (1951) and Colin and Verhoyen (1976). Because

of a lack of clear distinction between apical meristems and emerging leaf primordia, the measurement of shoot apices is problematical (Gifford, 1954). Schmidt (1924) included the first leaf buttress, whilst Popham and Chan (1950) and Rouffa and Gunckel (1951) excluded it. The most reliable method of determining width is recognition of the first divisions (generally periclinal), which localize the site of a new leaf buttress (Gifford, 1954). It is agreed that shoot apices should include the youngest defineable leaves (Gifford, 1954; Colin and Verhoyen, 1976).

Excisions of shoot apices sometimes consisted of apical meristems only (Smith and Murashige, 1970; Debergh and Maene, 1977; Shabde and Murashige, 1977), whereas in the majority of cases one to several leaf primordia of subapical regions were included (De Fossard, 1976; Murashige, 1977; Quak, 1977; Hussey, 1980; Allan, 1981a,b). Preference for the latter procedure depended on the type of plant species and purpose of research (Murashige, 1977; Walkey, 1980; Wang and Hu, 1980). The probability of recovering healthy plants from virus-infected stock is greater with the smallest explants (Limasett and Cornuet, 1949; Stone, 1963; Mori and Hosokawa, 1977; Quak, 1977; Walkey, 1980; Wang and Hu, 1980).

In vitro growth may be affected by explant size. Kassanis and Varma (1967), using excisions of 0,1 mm of potato apices, reported that explants containing one leaf primordium each responded much better than those lacking leaf primordia. Carnation shoot

apices smaller than 0,2 mm were unlikely to grow, whilst those larger than 0,75 mm produced plants that still contained mottle virus (Stone, 1963). In rhubarb (Walkey, 1968), explants having less than 2 - 3 primordial leaves failed to grow. For general propagation purposes, apices with 3 - 4 leaf primordia are dissected (Hussey, 1980; Walkey, 1980). In order to eliminate systemic viral infections, apices should be as small as possible, depending on the plant species and virus type (Mellor and Stace-Smith, 1977; Quak, 1977; Hussey, 1980; Walkey, 1980; Wang and Hu, 1980).

2.2.3 Primary vascular differentiation

2.2.3.1 Procambium

Investigations on growing shoots of the Dicotyledoneae revealed an initial continuity between the sites of leaf primordia and the vascular system of the axis (Esau, 1942, 1943b; Gunckel and Wetmore, 1946; Miller and Wetmore, 1946; Lawalrée, 1948; Mc Gahan, 1955). As the leaf primordia are elevated upon their buttresses, procambium differentiation proceeds concomitantly with the procambium of the leaf traces. The differentiation of procambium in the successively younger internodes in the order of their appearance and its continuation with leaf primordia is referred to as acropetal differentiation of the procambium (Esau, 1942; Lawalrée, 1948; Mc Gahan, 1955). In *V. vinifera* Fournioux (1972) reported elongation and orientation of cells in an expected pathway of differentiation of the procambium basal to the leaf initials and prior to the emergence of leaf primordia.

2.2.3.2 Vascular tissue

At the levels of leaf initiation in vascular plants, phloem and xylem differentiation show contrasting developmental sequences (Esau, 1943a,b, 1945; Gunckel and Wetmore, 1946; Sterling, 1947; Sussex, 1955; Jacobs and Morrow, 1957, 1958). Phloem differentiation proceeds acropetally, whereas xylem differentiates initially in the leaf-primordium bases or basipetally to them and then progresses acropetally in the leaf and basipetally in the axis. The differentiation of phloem elements starts prior to that of xylem elements in the leaf-traces (Esau, 1943a, b, 1945; Gunckel and Wetmore, 1946; Sussex, 1955; Jacobs and Morrow, 1957, 1958).

Differentiation of vascular tissues in elongating shoots of V. vinifera and V. labrusca have been described in detail by Hegedüs, 1969; Fournioux, 1972 and Pratt, 1974.

2.2.4 Anatomical responses to growth-regulator treatments of detached grapevine shoot apices cultured in vitro

Continued growth of leaf-like structures, derived from fragmented grapevine shoot apices, was dependent on the presence of 6-benzylaminopurine (BAP) (Barlass and Skene, 1980). Other cytokinins and auxins, viz. zeatin, 1-naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) were ineffective in producing adventitious buds and elicited only short-lived responses. Histological examinations showed that the basal swellings of the leaf-like structures from which adventitious shoots arose were composed mainly of parenchymatous cells, vascular

tissue and cambium. Serial sections obtained from basal regions showed the vascular tissue to be continuous with the leaf veins. Bud initiation was observed to be associated with meristematic areas which had vascular connections with deeper-seated vascular/ cambial tissues (Barlass and Skene, 1980).

As regards the shoot-apex culture of apple, Abbott and Whiteley (1976) reported that kinetin promoted production of adventitious and axillary shoots. Histological examinations revealed that adventitious shoots arose from meristematic units and did not, at least initially, have any vascular connection with the main shoot axis. Early stages of axillary shoot growth, once released from apical dominance, were also illustrated.

The effect of auxin on xylem differentiation has been effectively demonstrated in studies of regeneration of severed xylem in an internode of Coleus (Jacobs, 1954). Tissue culture studies also demonstrated the requirement for auxin in xylem differentiation. Jablonski and Skoog (1954) reported that pith tissue from tobacco stems cultured in contact with another piece containing vascular strands, would undergo cell divisions. It was suspected that the nurse piece provided substances which evoked the cell division response. In another example of a nurse tissue experiment, Wetmore and Sorokin (1955) induced the differentiation of vascular tissue in a block of callus tissue. Experiments of Wetmore and Sorokin (1955) and of Chutter (1960) implied that a localized or oriented occurrence of auxin is critical in the differentiation of xylem. Besides the information on morpho-

genetic substances from shoot apices influencing xylem differentiation, there is little evidence about other transmitted effects (Leopold, 1964). Wetmore (1959) and Wetmore and Rier (1963) stated that high sugar concentrations in culture media encourage phloem differentiation.

2.3 Virus distribution

Viruses are not equally distributed in all tissues of systematically infected plants (Broadbent, 1964; Hackett, 1966; Kassanis, 1967; Mellor Stace-Smith, 1977; Quak, 1977; Walkey, 1980; Wang and Hu, 1980). In the grapevine the uneven distribution of tomato ringspot virus (TomRSV) was demonstrated by Gonsalves (1982). If compared with other parts of the plant, many viruses are not found in high concentrations in meristematic tissues of root and shoot apices (Stone, 1963; Bajaj and Dionne, 1966; Walkey, 1978; Wang and Hu, 1980). White (1934) and Limasett and Cornuet (1949) indicated the existence of a concentration gradient of tobacco mosaic virus (TMV) in stems and roots, with virus content decreasing towards the apices. Elimination ratios of certain viruses were higher with very small (0,1 - 0,5 mm) excisions of shoot apices (Limasett and Cornuet, 1949; Stone, 1963; Hollings and Stone, 1968; Vine and Jones, 1969; Mori and Hosokawa, 1977; Quak, 1977; Walkey, 1980). Although cucumber mosaic virus (CMV) in lily, potato virus Y (PVY) and potato leaf roll virus (PLRV) in potato could be eliminated by culturing larger apices (0,5 - 3,0 mm), inactivation of tobacco mosaic virus (TMV) in petunia, potato virus X (PVX), potato virus G (PVG) and potato virus S (PVS) in potato depended on much smaller excisions (Mori and Hosokawa, 1977).

Viruses occasionally invade the shoot apex with the exception of the actual dome, but they sometimes even invade the latter (Broadbent, 1964; Hollings and Stone, 1964; Roberts et al., 1970; Mori and Hosokawa, 1977). For potato varieties no positive evidence could be offered for the absence of certain viruses in meristematic regions of shoot tips (Kassanis, 1957; Kassanis and Varma, 1967). Sheffield (1942) and Lackey (1946) reported the presence of TMV and curly top viruses at the tip of tobacco and tomato shoot meristems. Hollings and Stone (1964) proved the occurrence of carnation mottle viruses in the apical dome of the infected carnation plant. Walkey and Webb (1968, 1970), using electron microscopy, observed the presence of virus particles within the apical dome of Nicotiana rustica infected with Nepo viruses such as cherry leaf roll virus (CLRV) and strawberry latent ringspot virus (SLRV). Appiano and Pennazio (1972) detected particles of potato virus X (PVX) in shoot meristems of potato.

Reynolds and Corbett (1980) indicated the presence of viral-like particles in meristematic tissues of shoot tips of grapevine infected with tobacco ringspot viruses (TRSV). Barlass et al. (1982) stated that grapevine fanleaf viruses (GFLV) clearly are present in the young leaf primordia at the apex. The presence of certain viral-like particles in meristematic tissue may thus preclude the possibility of inactivation procedures by meristem or shoot tip culture (Reynolds and Corbett, 1980). Stellmach (1980a) obtained healthy plants from tender young shoot tips (1 - 5 cm) of vines infected with GFLV, arabis mosaic,

raspberry ringspot and tomato black ring viruses after forcing mother plants for 16 to 51 days at 30°C. This indicated that the distribution of GFLV or other Nepo viruses in infected vines growing in the field may be influenced by seasonal effects such as very warm conditions.

2.4 Virus inactivation from vegetatively propagated material

2.4.1 Inactivation by hot air treatments

Fulton and McGrew (1970) eliminated most strains of mottle-leaf virus in strawberries by treating infected plants at temperatures near 37°C for eight days to three weeks. Posnette and Cropley (1958), however, reported only a small proportion of strawberry plants cured from crinckle or yellow edge when held at 37°C for four to seven weeks. Heat-labile mosaic component viruses (HLMC) were inactivated in a number of British red raspberry varieties by growing dormant plants in lighted chambers at 35°C for five to 35 days (Chambers, 1961). Most plants were cured from leaf mottle virus but not from vein banding, vein chlorosis and yellows viruses and the varieties differed in their response to the treatment (Chambers, 1961).

Apple plants were cured from apple mosaic virus (AMV) within four weeks at 37°C (Posnette and Cropley, 1956) and cherry and peach plants from stone fruit ringspot virus within two and three weeks respectively (Nyland, 1960). Rives (1970) reported the complete cure of an entire plant of V. rupestris cv. Rupestris du Lot from GFLV by thermotherapy (163 days at 38°C). Among other ornamental plants cured from viruses by heat treat-

ment are carnation - from ringspot virus (Hollings, 1962), and rose - from mosaic virus (Holmes, 1960).

Success of heat treatment is largely determined by the chances of survival of the treated material (Baker, 1962; Van der Meer, 1967; Quak, 1977). Temperatures in the range of 25 - 30°C are generally considered optimal for both photosynthesis and growth in grapevines (Buttrose, 1969; Kriedemann and Smart, 1971). Higher temperatures over prolonged periods often result in diminishing shoot growth, which may be due to impaired growth *per se*, and/or declining of the roots (Kriedemann *et al.*, 1976). Some workers have also reported that fluctuating temperatures are more favourable for plant survival than constant temperatures (Mellor and Fitzpatrick, 1961; Larsen, 1974; Walkey and Freeman, 1977).

2.4.2 Inactivation by shoot apex culture alone and combined with thermotherapy

Culturing shoot apices or even larger shoot tips have eliminated virus pathogens and improved the quality and productivity of many species, including carnations (Baker and Phillips, 1962; Labrun-Peremans, 1966; Stone, 1968), cassava (Berbee *et al.*, 1973; Kartha *et al.*, 1974), chrysanthenum (Hill, 1968; Earle and Langhans, 1974), gladiolus (Simonsen and Hildebrandt, 1971), grapevines (Barlass *et al.*, 1982), orchids (Morel, 1960, 1964c; Kim, 1969), pelargonium (Pillai and Hildebrandt, 1969; Abo El-Nil and Hildebrandt, 1971, 1973, 1974, 1976), poplar (Winton, 1970; Berbee *et al.*, 1972), potato (Morel and Martin, 1955; Kumar, 1963; Svobodova, 1966; Chandra and Hildebrandt, 1967;

Christensen, 1968; Stace-Smith and Mellor, 1968a,b; Simonsen and Hildebrandt, 1972; Wang and Huang, 1975) and sugar cane (Heinz et al., 1969).

Thermotherapy combined with shoot apex culture enhanced the elimination rates of many viruses, e.g. carnation ring spot virus (Stone, 1968); leaf mottling viruses in chrysanthenum (Hollings and Stone, 1970); grapevine fanleaf virus (Gifford and Hewitt, 1961); necrotic ring spot virus in hops (Adams, 1975); raspberry ring spot and tomato black ring viruses in peach (Jacob, 1974); potato virus Y (Thomson, 1956) and strawberry pallidosis, strawberry mottle and strawberry mild yellow edge (Mullin et al., 1974; Mullin et al., 1975).

Rooting young shoot tips or culturing excised shoot apices in vitro after infected grapevines had been grown under sustained high temperatures (37°C and above) proved successful for the inactivation of several viruses (Goheen et al., 1965; Nyland and Goheen, 1969; Goheen and Luhn, 1973; Bovey, 1980; Stellmach, 1980a,b). The duration of thermotherapy varied with different viruses (Nyland and Goheen, 1969). Grapevine fanleaf, vein banding and yellow mosaic viruses were inactivated after 30 days at 38°C, followed by rooting shoot tips (1 - 5 cm) (Goheen et al., 1965). In employing the latter procedure for the inactivation of corky bark and leaf roll viruses, an extended period of heat treatment (up to 120 days) was necessary (Goheen et al., 1965; Nyland and Goheen, 1969).

It was stated (Rives, 1970; Bovey, 1980; Stellmach, 1980b; Wand and Hu, 1980) that virus multiplication may proceed within tissues of the plant that were not completely cured from the virus due to a lack of the necessary temperature-time-treatment. The *in vitro* application of heat treatment to rooted grapevine shoots (Galzy, 1964) allowed the provision of the necessary temperature conditions to the whole plantlet. An alternative approach is the application of heat treatment during *in vitro* culturing unrooted shoot apices, thus reducing the size of affected tissue subjected to thermotherapy (Wang and Hu, 1980). The latter procedure was successfully applied for the inactivation of cucumber mosaic and alfalfa mosaic viruses from infected shoot apices of *Nicotiana rustica* (Walkey and Cooper, 1975). The proliferation rate of adventitious shoots from fragmented grapevine shoot apices increased at a high temperature (35°C), in contrast to cultures at 27°C (Barlass *et al.*, 1981). In addition, GFLV could also be inactivated (Barlass *et al.*, 1982).

Since the elimination of viruses through shoot apex cultures does not result in immunity, it is essential to propagate the healthy plants under conditions that avoid reinfection (Quak, 1977; Stace-Smith, 1977; Walkey, 1980; Wang and Hu, 1980). The speed of reinfection and the nature of preventive measures to be taken, are largely determined by the epidemiology of the viruses involved (Quak, 1977). Obviously, reinfection is fully excluded as long as the plantlets remain under aseptic conditions. It is, therefore, advantageous to maintain stock materials, as well as to perform mass propagation *in vitro* (Boxus, 1964; Quak, 1977; Walkey, 1980; Wang and Hu, 1980).

SECTION 3

GENERAL EXPERIMENTAL PROCEDURES

3.1 Material

For all experiments described in sections 4, 5 and 6, material was sampled from Chenin blanc (*V. vinifera* L.) clone VIII/1 growing in a vineyard at the Oenological and Viticultural Research Institute near Stellenbosch and from the rootstock cultivar US 1-9 (Jacquez x Richter 99) (*V. aestivalis* M. x *V. cinerea* E. x *V. vinifera* L.) x (*V. berlandieri* P. x *V. rupestris* S.) growing as mother vines in a vineyard on the Welgevallen Experimental Farm of the University of Stellenbosch. Henceforth Chenin blanc and US 1-9 will be referred to as Chenin and 1-9, respectively.

Dormant canes were collected from selected vines. Only vines visually free of symptoms induced by virus and virus-like diseases were used. The canes were cut into lengths of 40 cm, treated with 2% Captan and stored in sealed plastic bags at 2 - 3°C until used.

Upon removal from cold storage, the canes were sectioned into cuttings of 12 - 15 cm, each comprising two internodes with three buds. The basal buds were removed. These cuttings were placed in 250 mℓ beakers with the bases in ca 80 mℓ water. Activation of shoot primordia was enhanced at an ambient temperature of 27°C under constant illumination. Budburst, followed by rapid shoot elongation, occurred after five to eight days. Prelimin-

ary experiments indicated that most of the young shoot tips were free of fungi, which normally are the main contaminants in culture. Large quantities of elongating shoots could hereby be procured on a small bench area within a short period and virtually at any time of the year.

Shoot tips (15 mm) were removed from elongating shoots exceeding 40 mm in length. Following removal of some of the outer leaves, the tips were surface sterilized in a 0,5% sodium hypochlorite solution for 10 min and rinsed thrice in sterile distilled water. Shoot apices (0,75 - 1 mm) containing 2 - 3 leaf primordia were excised aseptically under a dissecting microscope fitted with an ocular micrometer. The explants were individually cultured in test tubes (25 x 250 mm) containing 20 mℓ of the test medium. On all occasions explants were orientated horizontically (180°) on the surface of the solidified agar medium. Tubes were stoppered with cotton wool and covered with tin-foil caps.

3.2 Culture medium

Explants were cultured on the high salt (RM-62) medium of Murashige and Skoog (1962) (Table 1). All the stock solutions were prepared by dissolving the constituents in distilled water and then making up to volume. The inorganic salt stock solutions were stored in a refrigerator at 4 - 5°C. The vitamin stock solution was prepared by dissolving the components in distilled water, making up to volume and storing in 10 mℓ aliquots in stoppered specimen tubes at sub-zero temperatures until used.

In order to obtain the complete medium, the stock solutions were combined in the correct proportions (Table 1). The following supplements were added: 30 g/ℓ sucrose, 100 mg/ℓ myo-inositol and 6,8 g/ℓ Difco-Bacto agar. Fresh growth-substance solutions were prepared as recommended by De Fossard (1976) and added at various concentrations on each occasion. The pH of the medium was adjusted to 5,7 with 1 N NaOH prior to autoclaving (121°C for 15 min).

3.3 Culture conditions

Explants were incubated in a walk-in temperature-controlled culture room, maintained at 27°C during a 16h light period and 24 - 25°C during an 8h dark period. The light source consisted of "Atlas" cool-white fluorescent tubes providing 3 500 lux at culture level.

3.4 Morphological responses of excised shoot apices

In vitro responses of explants to different growth substances were investigated.

3.4.1 Data recording

Data were recorded on: fresh mass of callus growth at basal cut surfaces of explants after the shoot tissue had been carefully excised; shoot elongation (length of primary shoots); total mass of shoots; shoot proliferation (mean number of newly formed axillary shoots > 5 mm); percentages of explants with proliferated shoots; leaf expansion (mean number of expanded leaves per explant with expanded leaves); percentages of

cultures with expanded leaves; percentage rooting; mean number, mass and length of roots per rooted explant.

3.5 Anatomical investigations

Anatomical responses of explants were studied at various intervals (days) after the start of culture. For light microscopy (LM) material was fixed in formalin-acetic acid-alcohol (FAA), infiltrated by means of a tertiary butanol-paraffin wax procedure according to the method of Johansen (1940) and sectioned with a rotating microtome. For scanning electron microscopy (SEM) material was fixed in glutaraldehyde, dehydrated in a graded acetone-water series and subjected to crytical-point drying with CO_2, after which it was studied with a SEM.

3.6 Inactivation of grapevine fanleaf virus disease (GFLV)

3.6.1 Material

Material was sampled from a *V. rupestris* cv. Rupestris du Lot vine growing in a vineyard on the Welgevallen Experimental farm of the University of Stellenbosch, showing typical fanleaf symptoms. Green cuttings were propagated in a mist-bed (Goheen and Nyland, 1971) and subsequently established in a sandy soil-type in plastic containers. The vines were nursed in a plastic tunnel until used. Dormant canes were subjected to cold storage as outlined in par. 3.1. From these, elongating shoots were obtained by forcing the buds at 27°C as described earlier (3.1).

3.6.2 Construction of heat chamber

Treatments were conducted in a used transfer chamber measuring 710 mm x 540 mm x 540 mm (Fig. 1). The chamber consisted of a wooden

framework with transparent glass at the sides and top with an open bottom. Access to the chamber was through a sliding frontal lid. An opening of 720 mm x 720 mm was made in an ordinary laboratory bench. This opening was covered with perspex of the same dimensions in which holes of 5 cm ϕ were drilled. The chamber was put onto the perspex. Shoots from potted plants were trained through the laboratory bench into the heat chamber. The ambient temperature of roots in the pots varied from 20 to 25°C.

Heat was provided by 3 x 200 W incandescent lamps. The temperature within the chamber was controlled by means of a thermostat connected to the lamps. Lighting in the laboratory was continuous and was supplied by ordinary fluorescent tubes of a low intensity. Except for the lamps in the chamber, no supplementary light was provided.

3.6.3 Virus detection

The Enzyme-Linked Immunosorbent Assay (ELISA) procedure was applied for the detection of GFLV in grapevine tissues following procedures to inactivate the virus.

The antiserum to GFLV was kindly provided by Dr J J Joubert of the Department of Microbiology and Virology of the University of Stellenbosch. Purification and conjugation of gamma-globulin, as well as the calibration of the polystyrene microtitre plates were carried out as described by Clark and Adams (1977). The concentration of gamma-globulin used for optimum coating of the wells was 2 g/mℓ. All tissues for ELISA tests were macerated

in phosphate-buffered saline solution (PBS) containing 0,05% Tween 20, plus 2% polivinyl-pyrrolidone (PVP) and 1% nicotine. In all tests conjugated gamma-globulin were diluted 1 : 800 in PBS with 0,05% Tween 20 (PBS-Tween) containing 2% PVP and 0,2% bovine serum albumin. Visual assessment of the contents of each well was recorded ca 1h after adding the enzyme substrate, p-nitrophenyl phosphate, to the wells; the reaction was stopped with NaOH.

Table 1 - Murashige and Skoog (1962) high salt medium: Composition of stock solutions

Bottle	Constituent	Stock solution (g/ℓ)	Dilution (mℓ/ℓ)	Final concentration in medium (mg/ℓ)
I	NH_4NO_3	82,5	20	1 650
II	KNO_3	95	20	1 900
III	$CaC\ell_2 \cdot 2H_2O$	44	10	440
IV	$MgSO_4 \cdot 7H_2O$	37	10	370
V	KH_2PO_4	17	10	170
VI	$FeSO_4 \cdot 7H_2O$	1,39	20	27,86
	Na_2 EDTA	1,86		37,26
VII	$MnSO_4 \cdot 4H_2O$	22,3	1	22,3
	$ZnSO_4 \cdot 7H_2O$	8,6		8,6
	$CuSO_4 \cdot 5H_2O$	0,025		0,025
VIII	H_3BO_3	6,2	1	6,2
	KI	0,83		0,83
	$Na_2MoO_4 \cdot 2H_2O$	0,25		0,25
	$CoC\ell_2 \cdot 5H_2O$	0,025		0,025
IX	Thiamine	0,02	5	0,1
	Nicotinic acid	0,1		0,5
	Pyridoxine	0,1		0,5
	Glycine	0,4		2,0

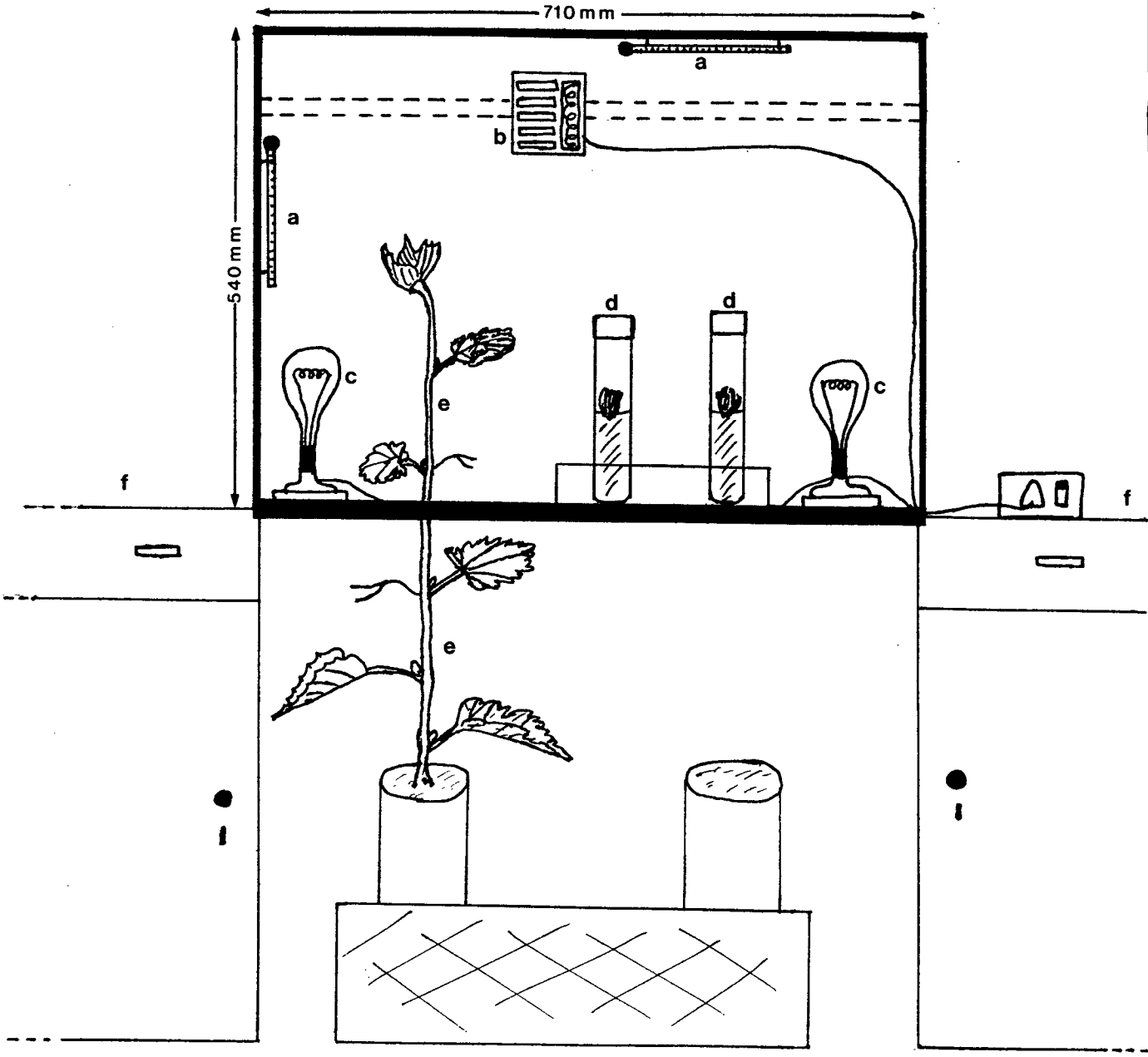


Fig. 1 - Frontal view of heat chamber: (a) thermometer; (b) thermostat; (c) incandescant lamp; (d) test tube with proliferated shoots; (e) diseased vine (Rup. du Lot) in plastic container with elongating shoot within chamber and (f) laboratory bench.

SECTION 4

RESPONSES TO PLANT GROWTH REGULATORS OF SHOOT APICES (0,75 - 1 mm) OF GRAPEVINES CULTURED IN VITRO

4.1 Introduction

Based on the findings of Skoog and Tsui (1948), Skoog and Miller (1957) and Wickson and Thimann (1958), morphological responses of detached shoot apices cultured *in vitro* could be manipulated through growth regulators provided in the nutrient medium, especially cytokinin and auxin.

Responses such as elongation of primary shoots, leaf expansion, growth of axillary shoots (proliferation) and the initiation and growth of callus and roots depend largely on the concentrations and ratios of these growth regulators when applied to *in vitro* cultures (Murashige, 1974, 1977; De Fossard, 1976; Sagawa, 1976; Hussey, 1980; Allan, 1981a,b). In grapevines, reports are limited as regards appropriate growth regulators and their concentrations for eliciting desired morphogenic responses.

The purpose of this study was: (i) to investigate responses of excised shoot apices to various growth regulators, whether applied singly or in combination, (ii) to determine optimal concentrations of growth regulators required for maximal responses of explants and (iii) to investigate to what extent these responses could be controlled by interactions between different growth regulators.

4.2 Effects of kinetin alone and in combination with auxins

4.2.1 Objective and procedure

This experiment was conducted to determine responses of detached shoot apices of Chenin and 1-9 to different concentrations of kinetin alone and in combination with indole-3-acetic acid (IAA), 1-naphthaleneacetic acid (NAA) and indole-3-butyric acid (IBA). The standard procedure (3.1, 3.2, 3.3) was followed and the treatments listed in Figs. 2, 3 and 4 were applied. Each treatment consisted of two replicates of 10 explants each, with a culture period of 56 days.

Data for each cultivar were subjected to analysis of variance followed by the Student Newmann-Keuls plural comparison test (Snedecor and Cochran, 1967).

4.2.2 Results

The results are presented in Figs. 2, 3, 4, 5, 6, 7, 8, 9 and 10, and Appendices 1.1, 1.2, 2.1, 2.2, 3.1 and 3.2. Unless otherwise indicated, results refer to both cultivars.

4.2.2.1 Shoot elongation

In the absence of auxins, elongation of primary shoots was stimulated by increasing concentrations of kinetin. Maximum elongation resulted at 8 mg/ℓ; it was significantly higher than that recorded at lower kinetin levels (0,25 - 1 mg/ℓ) and the control. In the absence of kinetin, elongation in Chenin was stimulated by IAA only and reached a maximum at 4 - 8 mg/ℓ; in 1-9 elongation was not significantly stimulated by IAA at all.

Application of the highest levels of NAA alone suppressed elongation; whereas IBA had no significant effect.

Combined applications of kinetin and IAA stimulated elongation the most. In the presence of 0,5 - 8 mg/ℓ IAA (Chenin) and 0,5 - 4 mg/ℓ IAA (1-9), length of primary shoots increased with increasing concentrations of kinetin. A maximum was reached with 8 mg/ℓ kinetin and 4 mg/ℓ IAA. The highest level of IAA (16 mg/ℓ) significantly suppressed elongation in 1-9 when combined with 0,25 - 8 mg/ℓ kinetin.

Combinations of kinetin (0,25 - 8 mg/ℓ) with increasing concentrations of NAA and IBA stimulated elongation if compared with control treatments (no kinetin). However, inhibitory effects of NAA and IBA were not totally overcome with combined applications of kinetin (even at 8 mg/ℓ).

4.2.2.2 Shoot mass

In both cultivars, shoot mass increased in response to kinetin treatments, reaching a maximum at 8 mg/ℓ. In combination with IAA, maximum increase in mass resulted with 8 mg/ℓ kinetin and 4 mg/ℓ IAA (significantly higher than that recorded for kinetin alone at 8 mg/ℓ). Combined applications of NAA or IBA (0,1 - 8 mg/ℓ) with kinetin (0,25 - 8 mg/ℓ) resulted in lower mass increases than that induced by kinetin alone at each level.

4.2.2.3 Callus growth at basal cut surfaces of explants

No callus growth occurred in the presence of kinetin alone.

Application of IAA, singly as well as in combination with kinetin, also failed to induce callus growth.

In the absence of kinetin, only NAA, of the auxins tested, induced callus growth. Combined applications of kinetin and NAA (1 mg/ℓ kinetin with 0,5 - 2 mg/ℓ NAA and 2 mg/ℓ kinetin with 0,5 - 4 mg/ℓ NAA) resulted in significantly higher callus masses in 1-9 than those recorded for other treatments. Callus growth was, however, strongly suppressed at higher concentrations of kinetin and NAA in combination. Although in Chenin the highest callus masses occurred with 0,25 mg/ℓ kinetin in combination with 4 mg/ℓ NAA, it was not significantly better than that recorded for NAA alone. Low concentrations of NAA (0,1 mg/ℓ) induced callus growth only when combined with 2 - 4 mg/ℓ kinetin.

In the absence of kinetin (or at 0,25 mg/ℓ kinetin), callus growth could not be induced by IBA whilst it was stimulated in the presence of kinetin. Maximum callusing resulted with 8 mg/ℓ IBA in combination with 4 and 2 mg/ℓ kinetin (Chenin and 1-9, respectively). However, no callus growth occurred in either Chenin or 1-9 at higher kinetin concentrations (8 mg/ℓ) in combination with the IBA levels employed.

4.2.2.4 Leaf expansion

Numbers of expanded leaves increased with increasing concentrations of kinetin and reached a maximum at 8 mg/ℓ. In the absence of kinetin, no leaf expansion occurred regardless of the auxin concentration tested.

Combined applications of kinetin and IAA induced more expanded leaves than kinetin alone. However, at 16 mg/ℓ (Chenin) and at 8 and 16 mg/ℓ (1-9), IAA caused a reduction in leaf expansion when combined with kinetin at different levels. Combinations of 4 and 8 mg/ℓ kinetin and 0,1 mg/ℓ NAA induced expanded leaves in Chenin and at 8 mg/ℓ kinetin with 0,1 - 0,5 mg/ℓ NAA in 1-9. Inhibitory effects of NAA at concentrations exceeding 0,1 mg/ℓ (Chenin) and 0,5 mg/ℓ (1-9) were not cancelled by adding kinetin (even up to 8 mg/ℓ).

4.2.2.5 Percentages of explants with expanded leaves

Kinetin alone induced leaf expansion in all cultures (100%) at the highest concentrations applied (4 and 8 mg/ℓ). However, leaf expansion in 100% of the cultures could be achieved with lower concentrations of kinetin (2 mg/ℓ) when applied in combination with appropriate IAA levels. In the presence of NAA, kinetin failed to induce leaf expansion in all cultures. Combinations of kinetin (4 and 8 mg/ℓ) with IBA (0,1 - 1 mg/ℓ for Chenin and 0,1 - 0,5 mg/ℓ for 1-9) resulted in leaf expansion in 100% of the cultures.

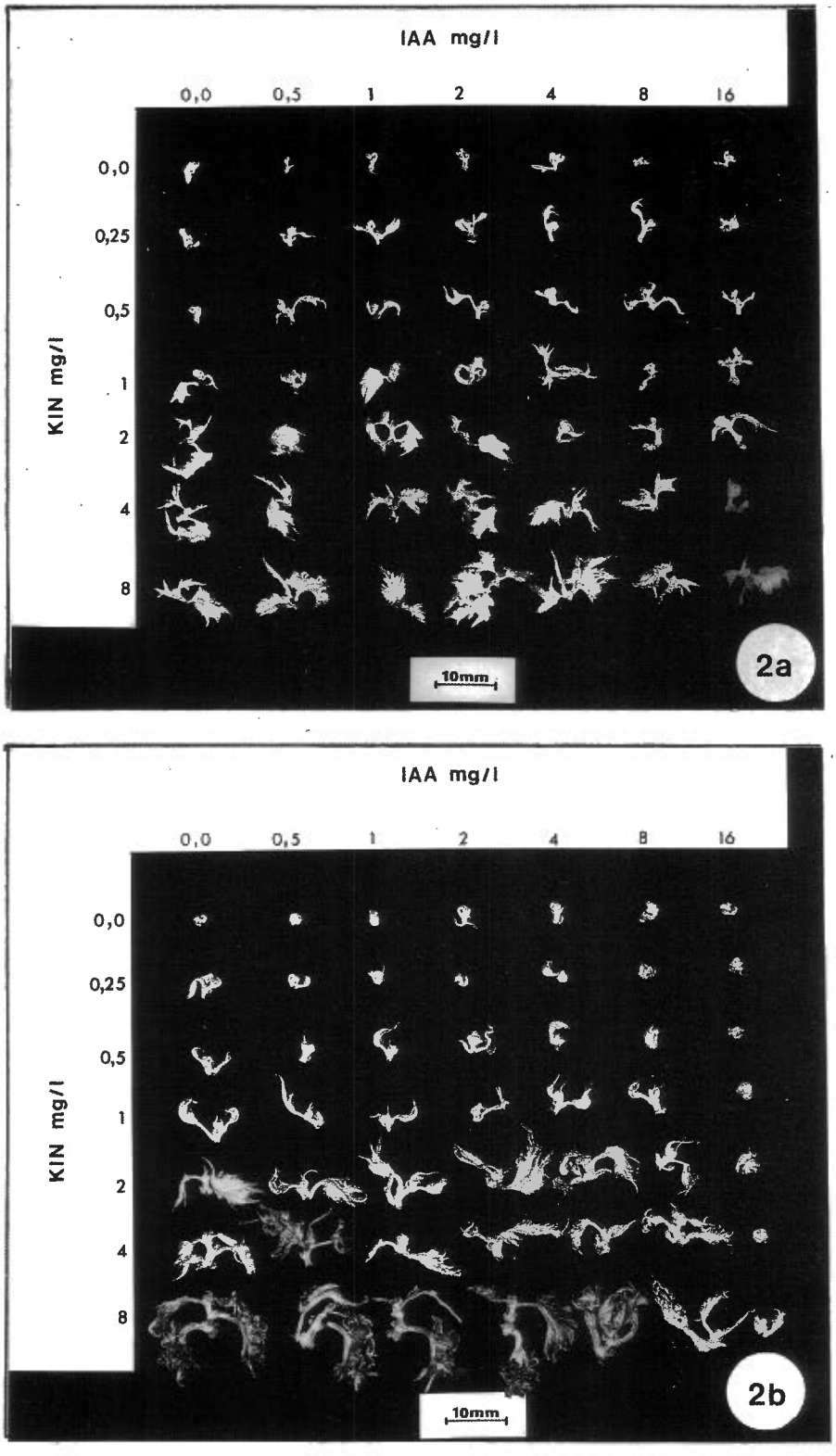


Fig. 2 – In vitro growth responses of excised shoot apices of Chenin (a) and 1-9 (b) to different concentration ratios of kinetin and indole-3-acetic acid (IAA)

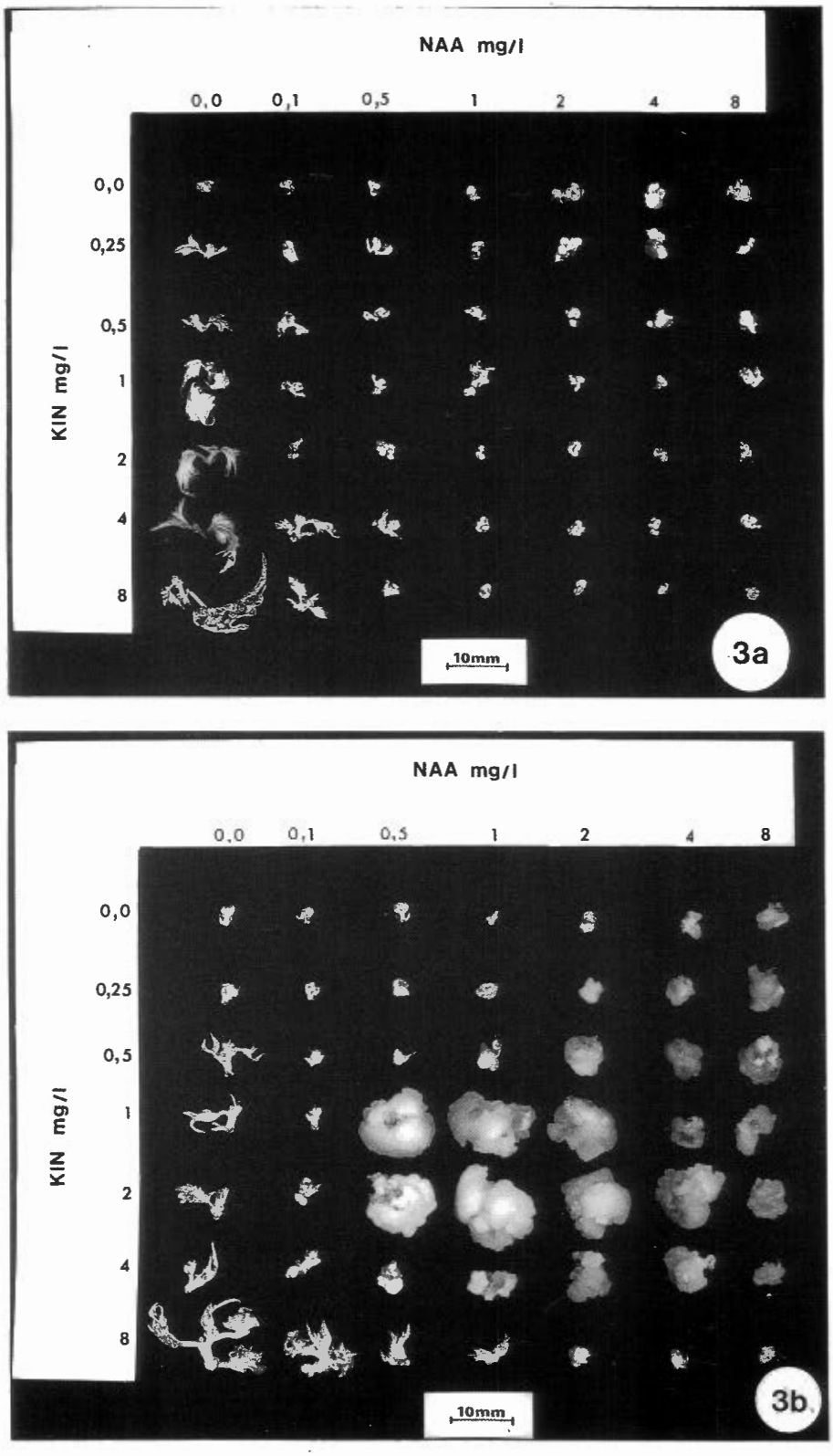


Fig. 3 - In vitro growth responses of excised shoot apices of Chenin (a) and 1-9 (b) to different concentration ratios of kinetin and 1-naphthaleneacetic acid (NAA)

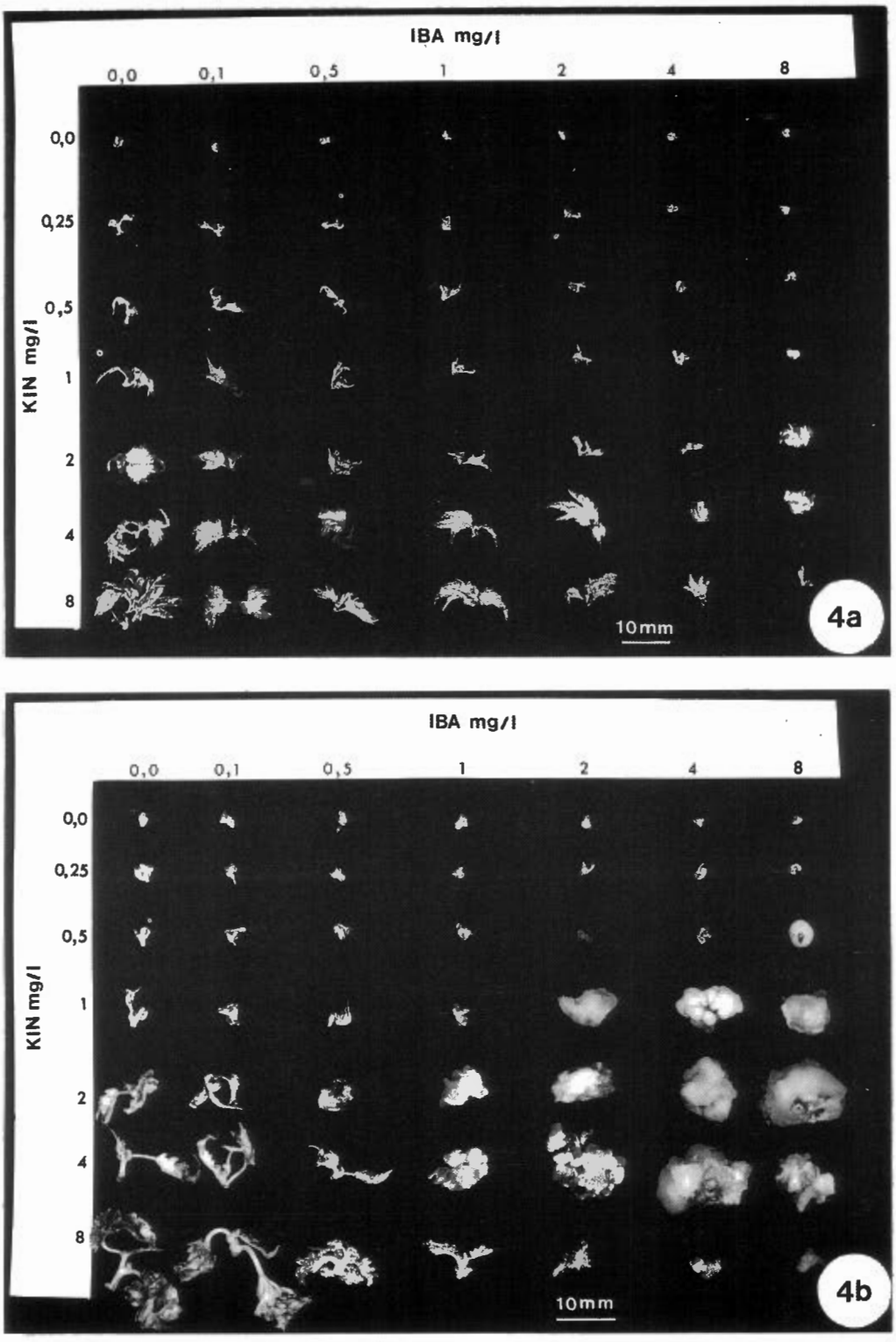


Fig. 4 -- In vitro growth responses of excised shoot apices of Chenin (a) and 1-9 (b) to different concentration ratios of kinetin and indole-3-butyric acid (IBA)

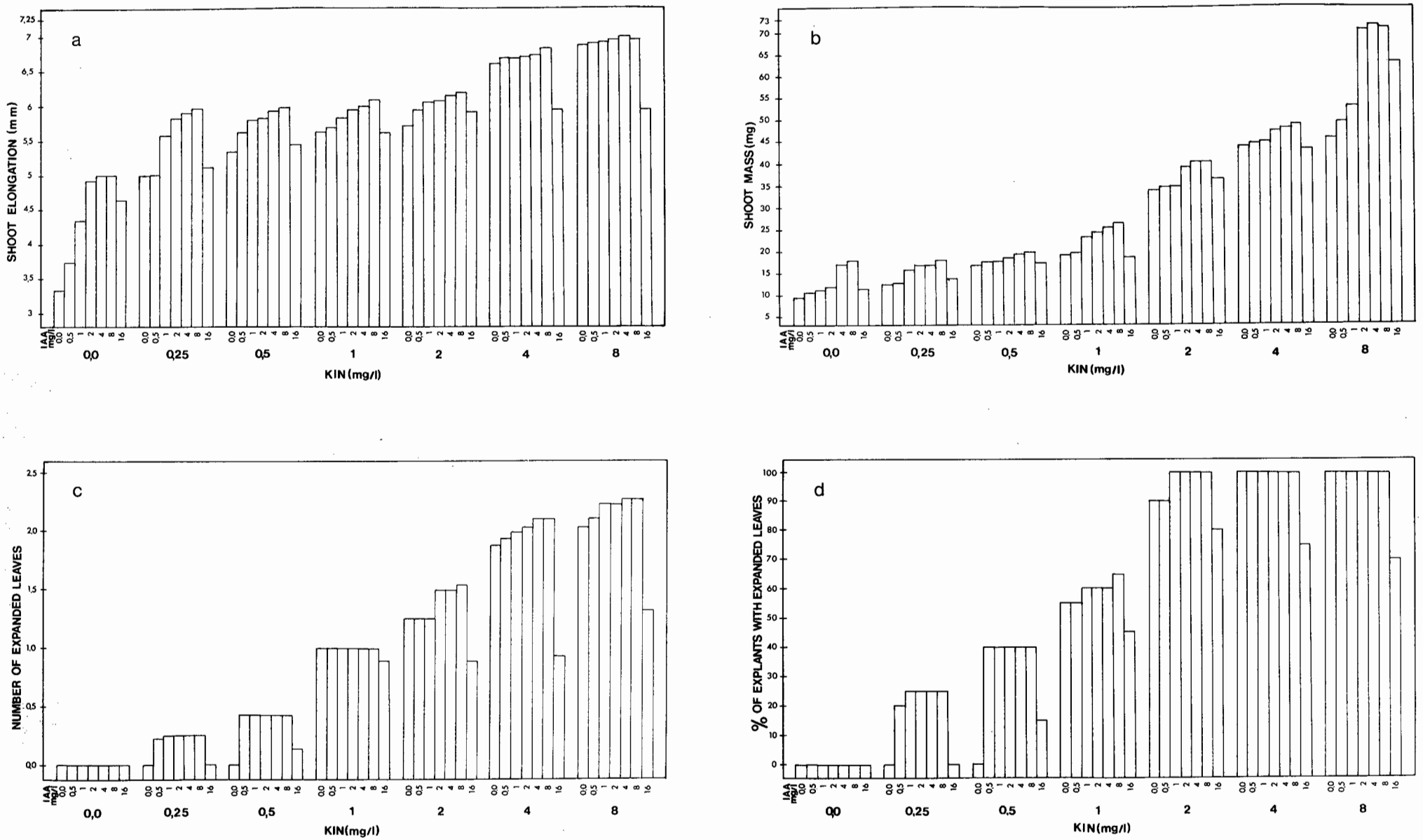


Fig. 5 - The effect of different concentration ratios of kinetin and indole-3-acetic acid (IAA) on shoot elongation (a), total shoot mass (b), number of expanded leaves (c), and percentages of explants with expanded leaves (d), derived from shoot apices of Chenin cultured in vitro

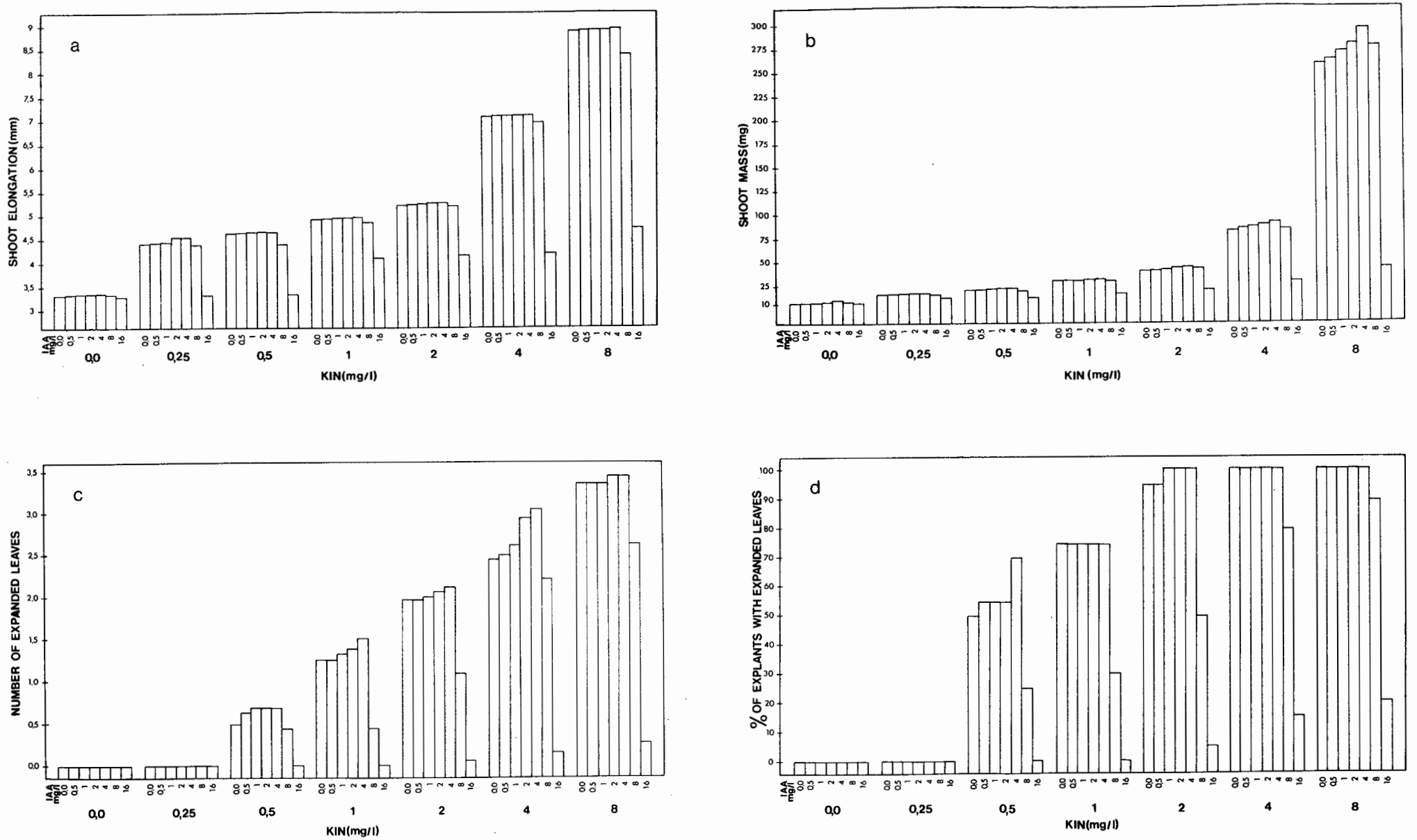


Fig. 6 - The effect of different concentration ratios of kinetin and indole-3-acetic acid (IAA) on shoot elongation (a), total shoot mass (b), number of expanded leaves (c), and percentages of explants with expanded leaves (d), derived from shoot apices of 1-9 cultured in vitro

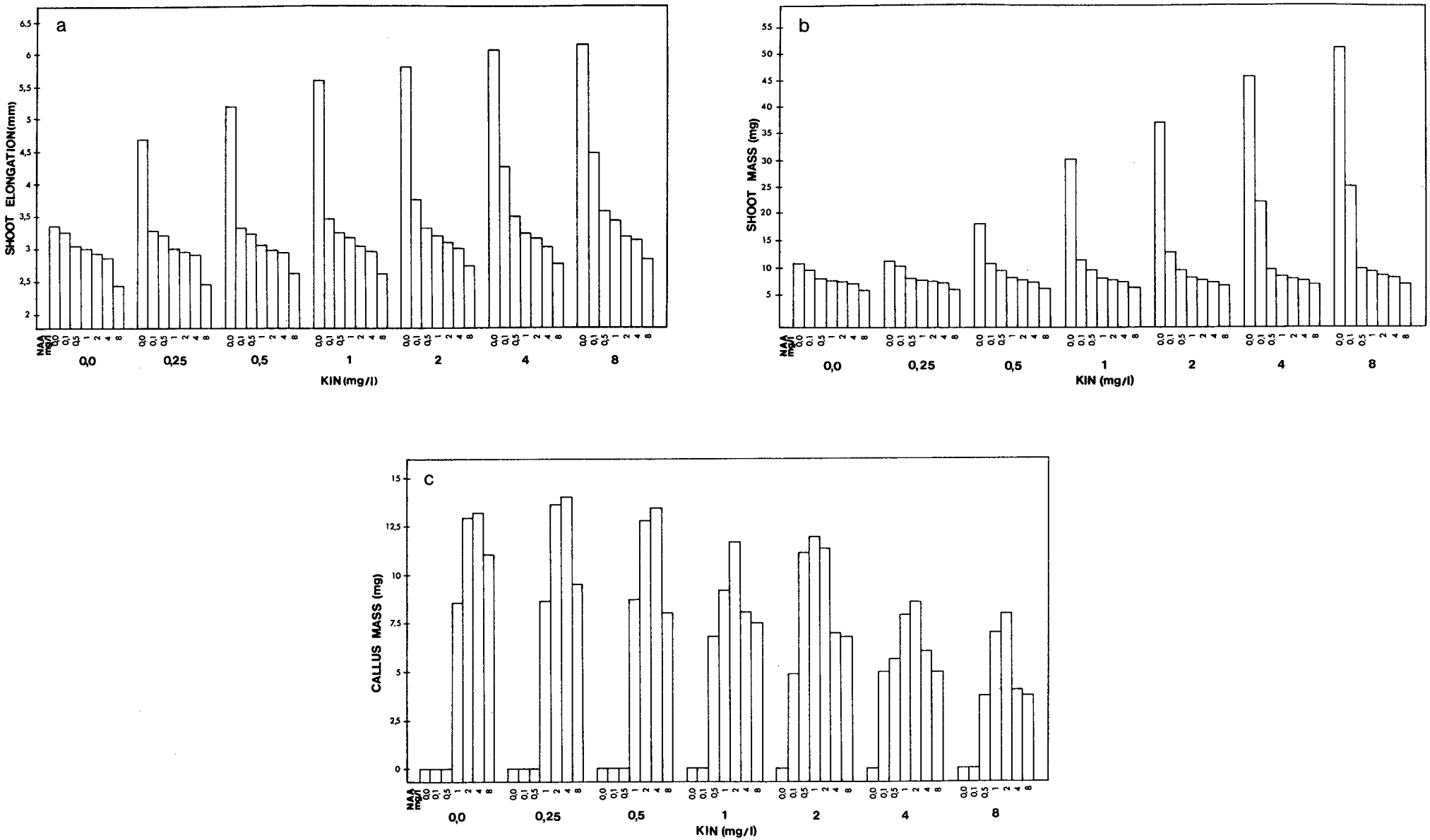
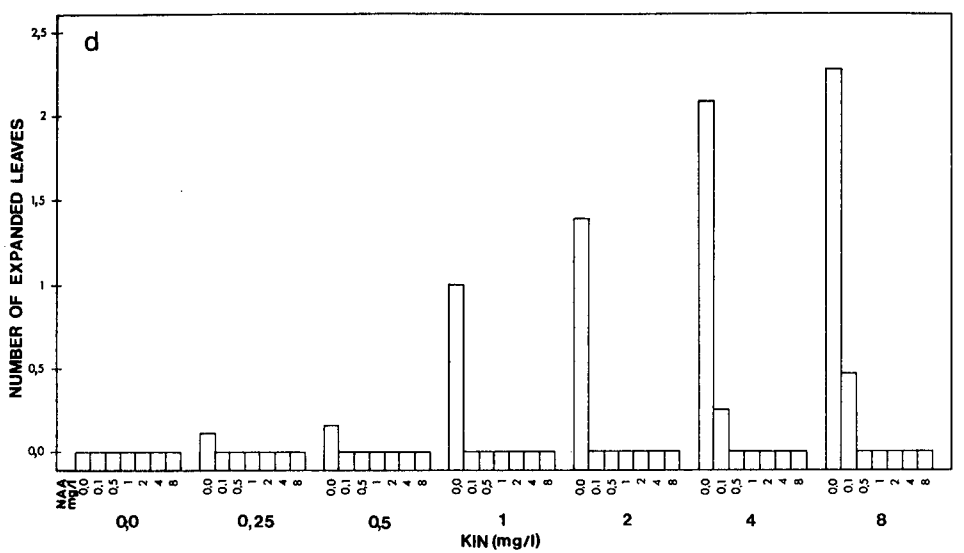


Fig. 7 (a-c) - The effect of different concentration ratios of kinetin and 1-naphthaleneacetic acid (NAA) on shoot elongation (a), total shoot mass (b), and callus mass (c), derived from shoot apices of Chenin cultured in vitro



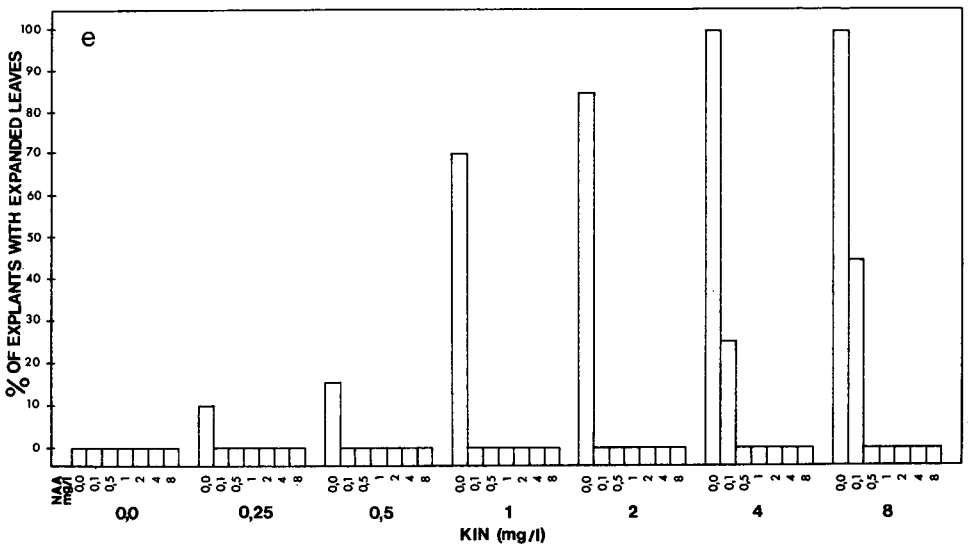


Fig. 7 (d-e) - The effect of different concentration ratios of kinetin and 1-naphthaleneacetic acid (NAA) on number of expanded leaves (d), and percentages of explants with expanded leaves (e), derived from shoot apices of Chenin cultured in vitro

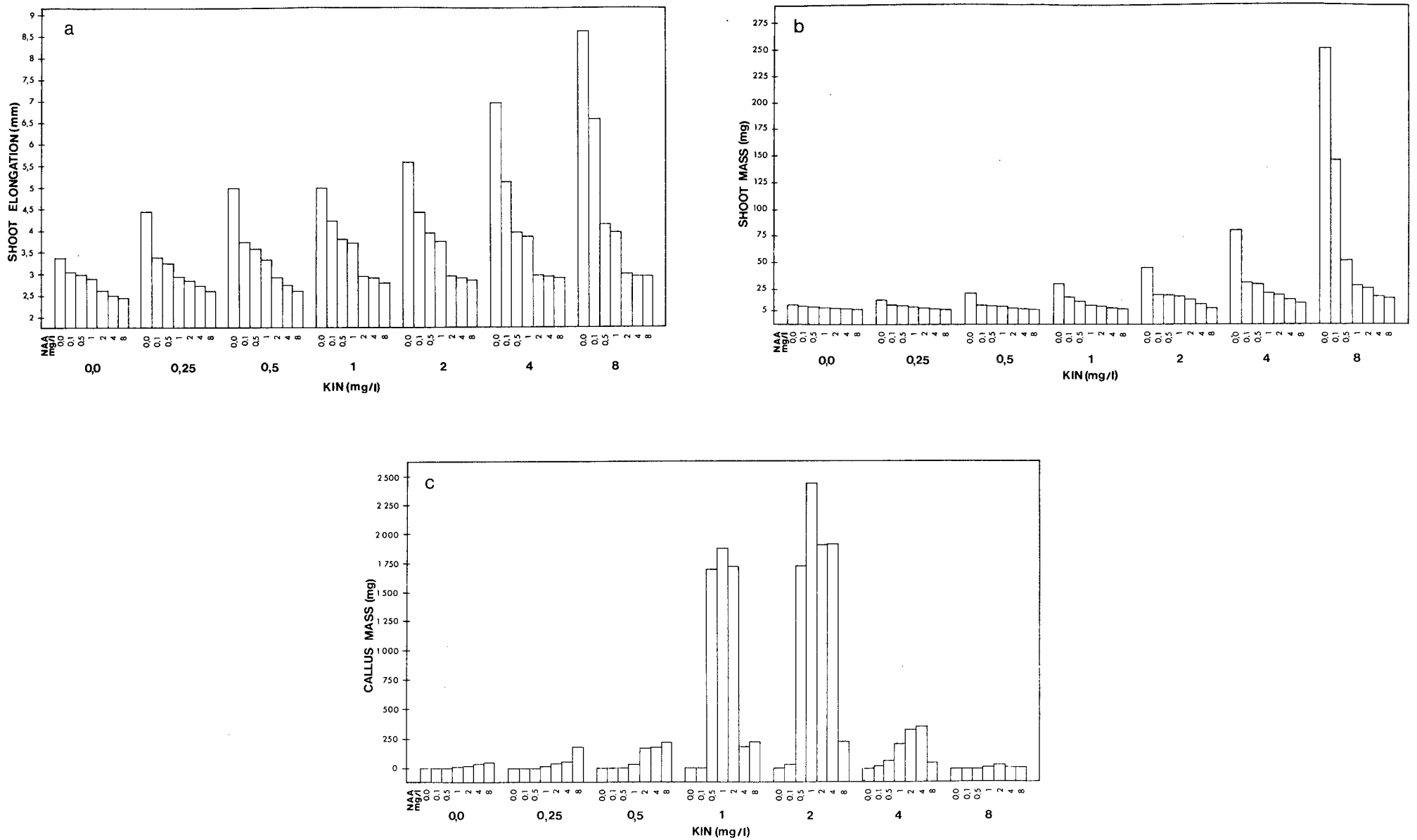
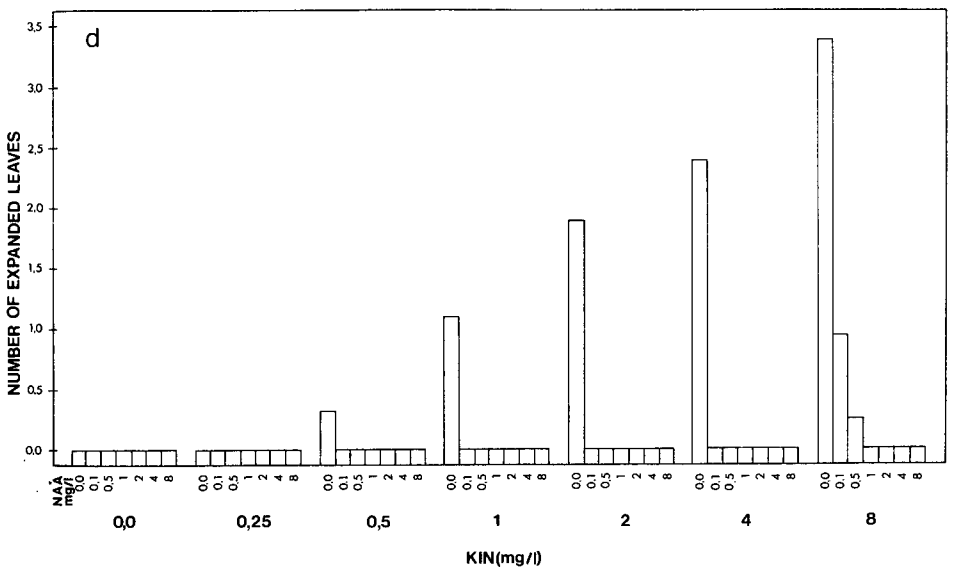


Fig. 8 (a-c) - The effect of different concentration ratios of kinetin and 1-naphthaleneacetic acid (NAA) on shoot elongation (a), total shoot mass (b), and callus mass (c), derived from shoot apices of 1-9 cultured in vitro



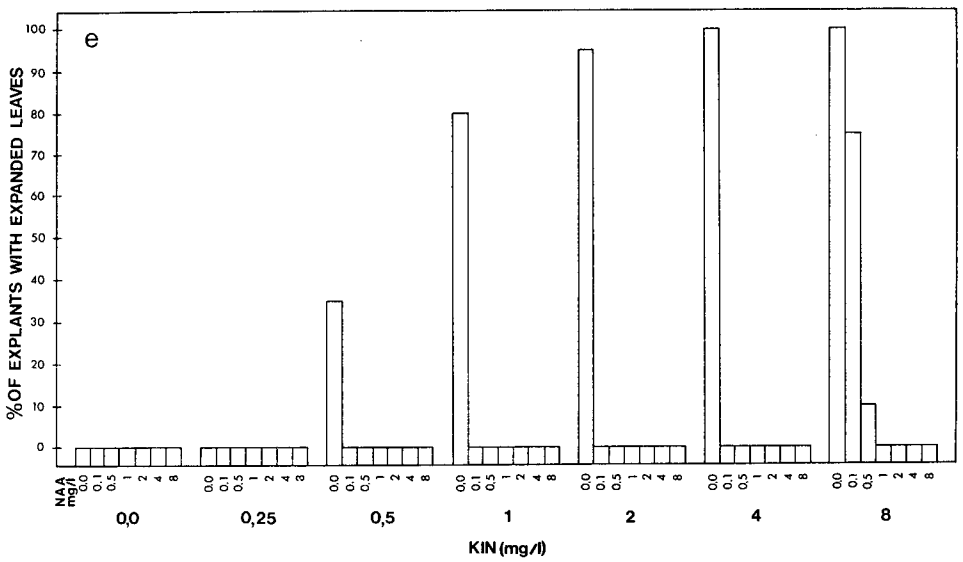


Fig. 8 (d-e) - The effect of different concentration ratios of kinetin and 1-naphthaleneacetic acid (NAA) on number of expanded leaves (d), and percentages of explants with expanded leaves (e), derived from shoot apices of 1-9 cultured in vitro

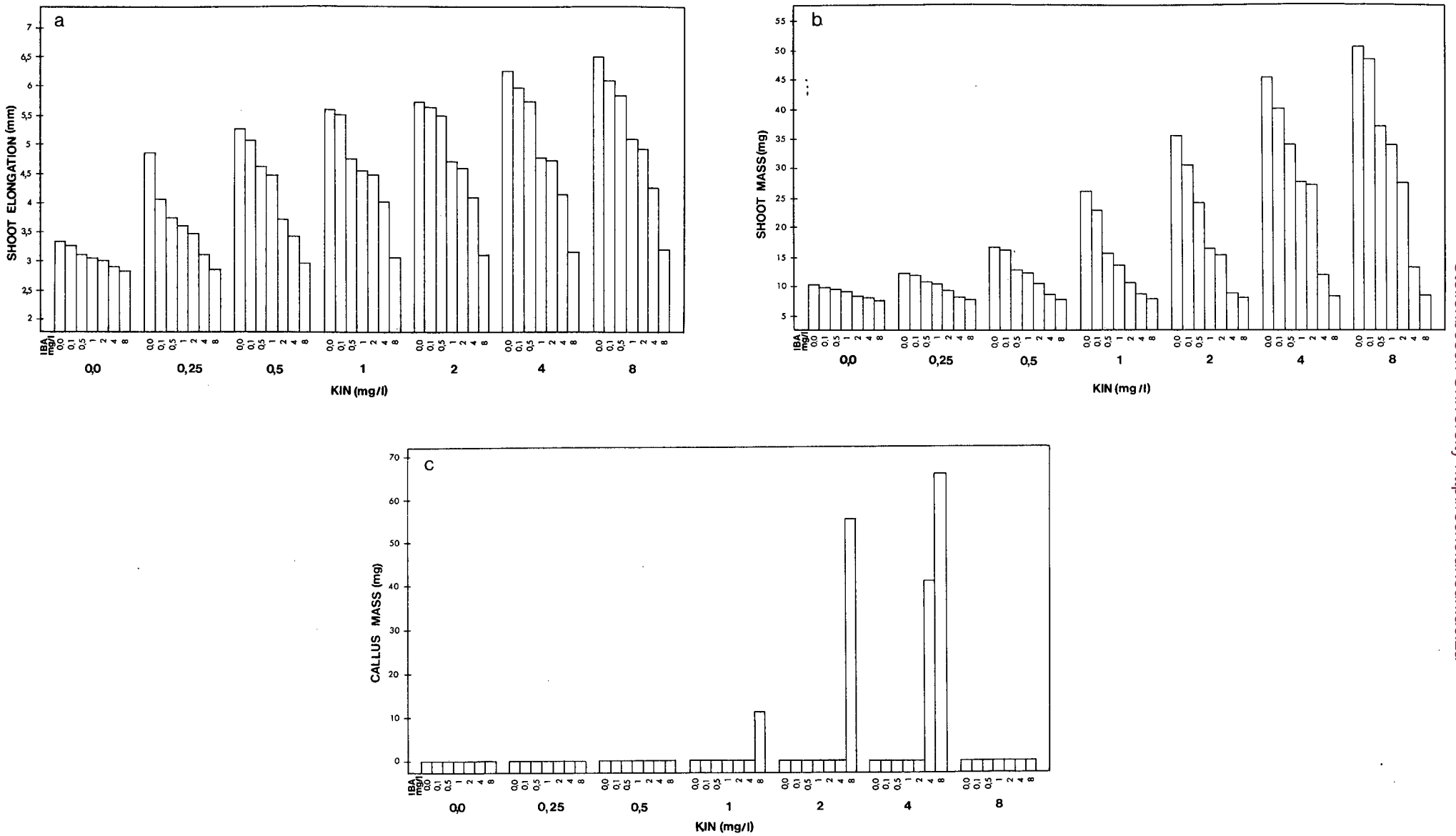
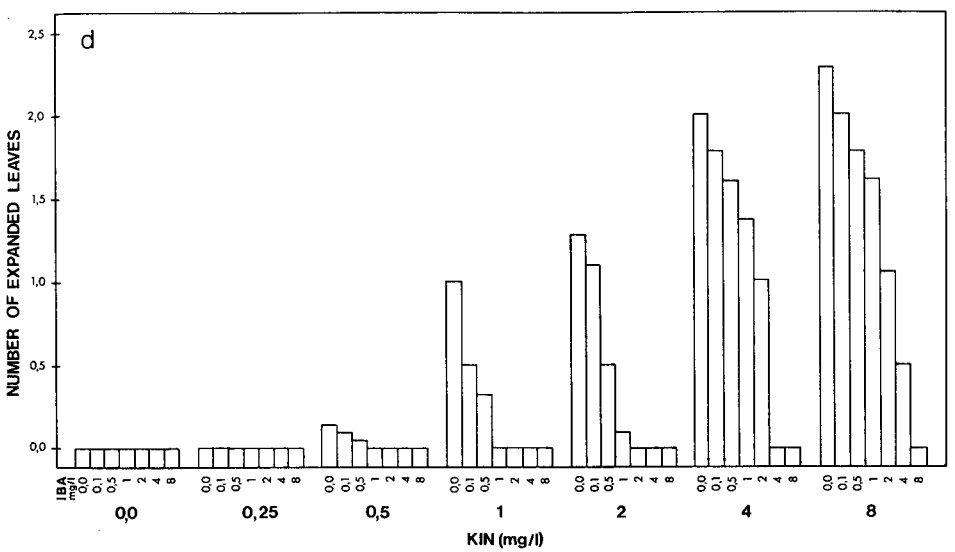


Fig. 9 (a-c) - The effect of different concentration ratios of kinetin and indole-3-butyric acid (IBA) on shoot elongation (a), total shoot mass (b), and callus mass (c), derived from shoot apices of Chenin cultured in vitro



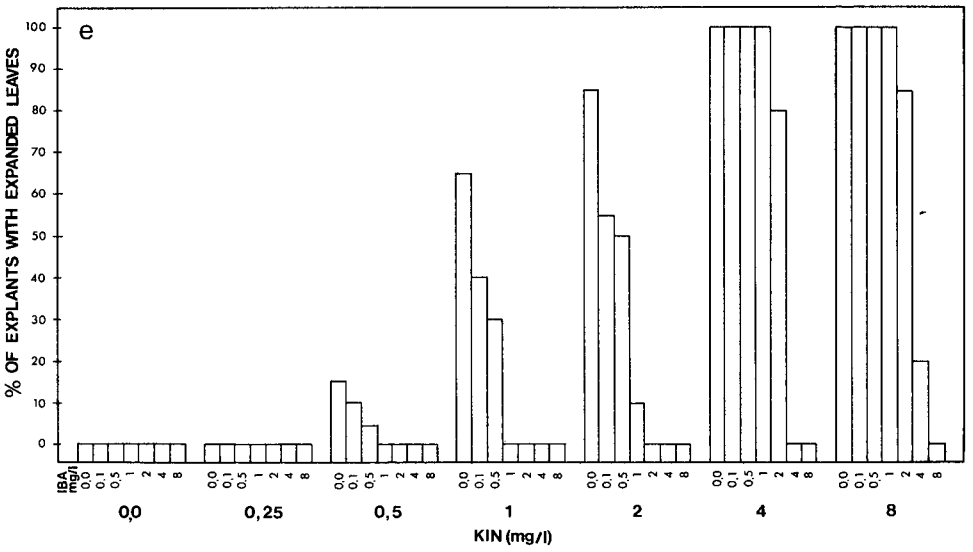


Fig. 9 (d-e) - The effect of different concentration ratios of kinetin and indole-3-butyric acid (IBA) on number of expanded leaves (d), and percentages of explants with expanded leaves (e), derived from shoot apices of Chenin cultured in vitro

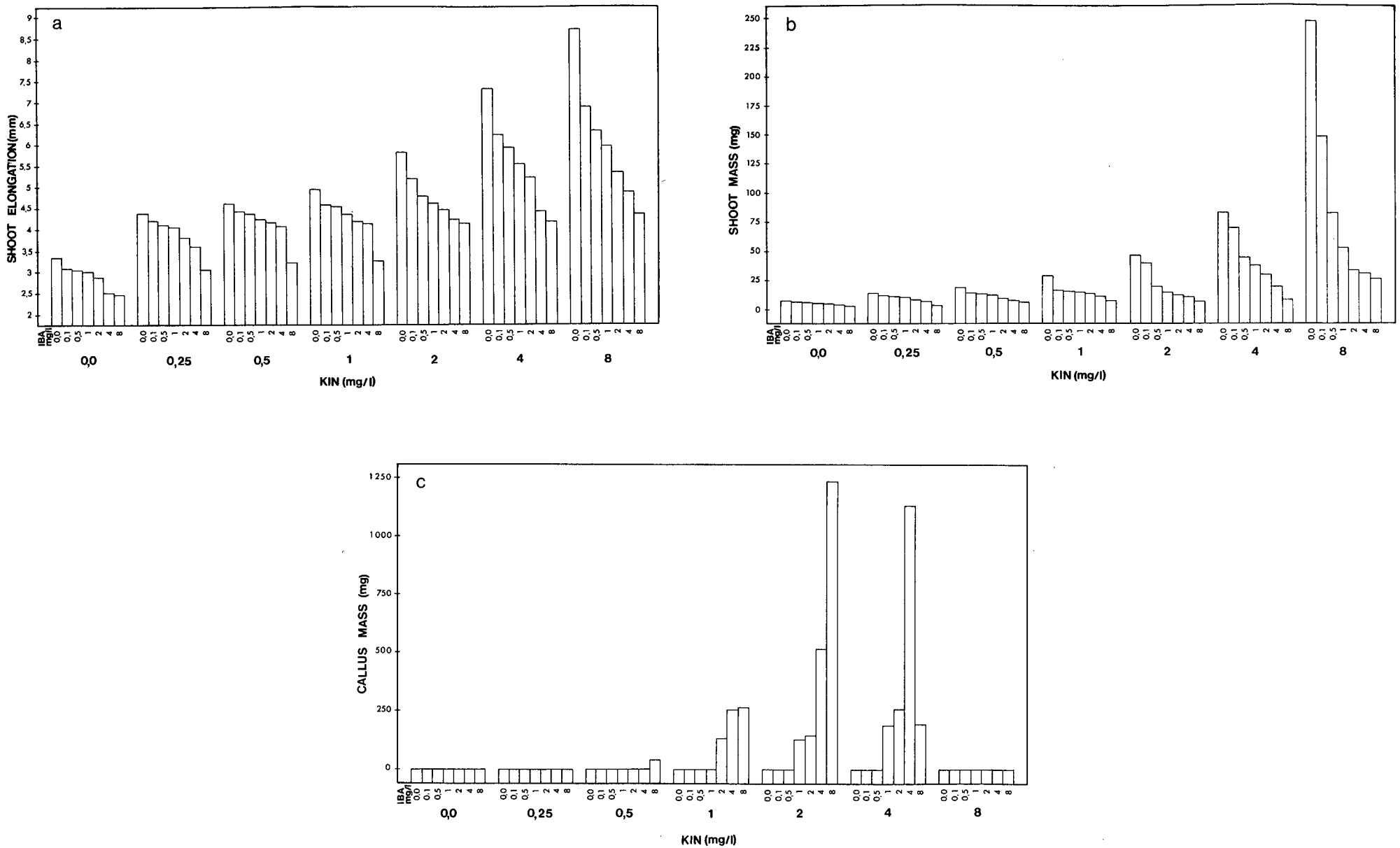
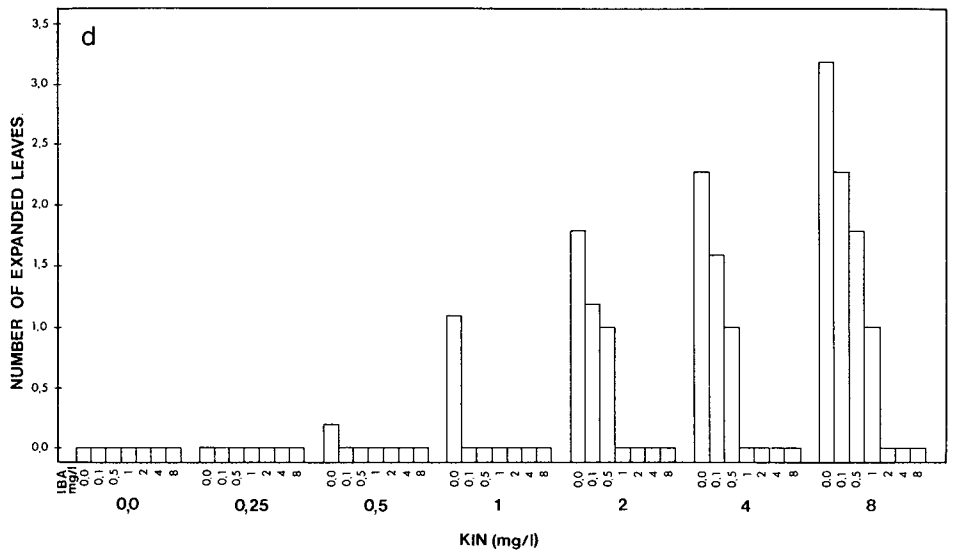


Fig. 10 (a-c) - The effect of different concentration ratios of kinetin and indole-3-butyric acid (IBA) on shoot elongation (a), total shoot mass (b), and callus mass (c), derived from shoot apices of 1-9 cultured in vitro



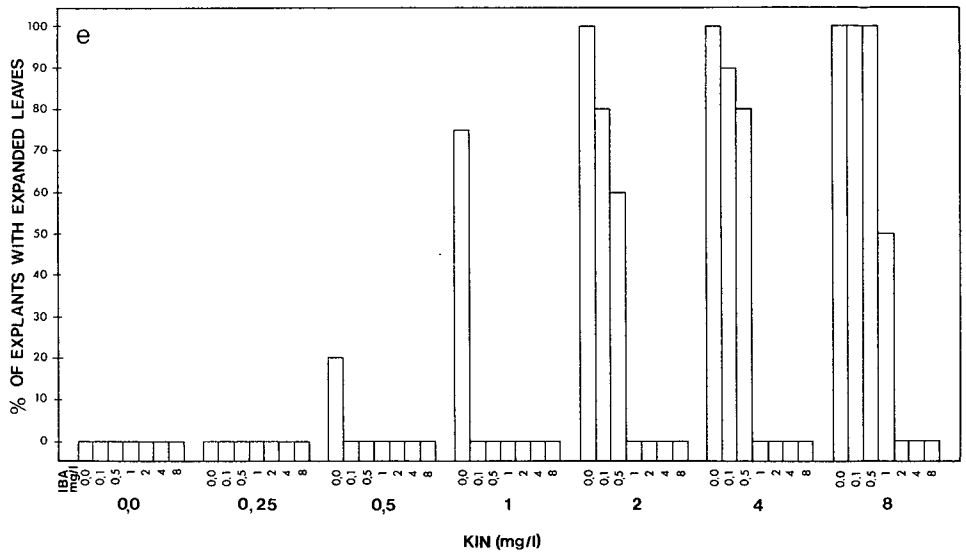


Fig. 10 (d-e) - The effect of different concentration ratios of kinetin and indole-3-butyric acid (IBA) on number of expanded leaves (d), and percentages of explants with expanded leaves (e), derived from shoot apices of 1-9 cultured in vitro

4.3 Effects of different concentrations of cytokinins

4.3.1 Objective and procedure

The optimal concentration of cytokinin for eliciting maximal responses in shoot apex cultures varies greatly from species to species (Murashige, 1977; Cheng, 1978; Hussey, 1978, 1980; Anderson, 1980). In the previous experiment kinetin stimulated shoot elongation and leaf expansion. However, no axillary shoots were produced, even at 8 mg/ℓ, thus indicating that higher concentrations might be needed. This experiment was conducted to compare the responses of excised shoot apices of Chenin and 1-9 to different concentrations of four different cytokinins, viz., kinetin, 6-benzylaminopurine (BAP), zeatin and zeatin riboside (ZR) shown in Fig. 11. The standard procedure (3.1, 3.2, 3.3) was followed and the culture period extended over 30 days. Each treatment included three replicates of 10 explants each. Data for each cytokinin were subjected to analysis of variance followed by the Duncan's plural comparison test (Snedecor and Cochran, 1967).

4.3.2 Results

The results are presented in Figs. 11, 12, 13, 14 and 15, and Appendices 4.1, 4.2, 4.3 and 4.4. Unless otherwise indicated, results refer to both cultivars.

4.3.2.1 Shoot elongation

Primary shoot length increased almost linearly in response to increasing levels of kinetin. Maximum elongation resulted at 20 mg/ℓ; it was significantly higher than that recorded for

lower levels (0,1 - 10 mg/ℓ for Chenin and 0,1 - 15 mg/ℓ for 1-9). BAP proved the most effective at 2 mg/ℓ (significant at the 5% level). However, at higher concentrations (> 2 mg/ℓ) elongation was either suppressed (1-9) or inhibited (Chenin).

Shoot elongation was greatly stimulated by zeatin treatments, reaching maximum levels at 15 mg/ℓ. A suppression followed at 20 mg/ℓ (significantly lower than recordings for 15 mg/ℓ). Excised shoot apices responded markedly to the addition of ZR. Maximum elongation resulted at 10 mg/ℓ. However, at higher concentrations (15 - 20 mg/ℓ) elongation was suppressed.

4.3.2.2 Shoot mass

Maximum increase in mass in response to individual cytokinins was achieved at the same concentrations that were most stimulatory for shoot elongation.

4.3.2.3 Shoot proliferation

Shoot proliferation rates were controlled to a high extent by different concentrations of the individual cytokinins. For kinetin highest numbers of proliferated shoots resulted at the highest concentrations tested (20 mg/ℓ). BAP proved the most effective at 2 mg/ℓ, but a complete inhibition resulted at higher levels. With zeatin, the maximum proliferation rate was obtained at 15 mg/ℓ, whereas ZR induced the highest number of shoots at 10 mg/ℓ. Higher levels of ZR were inhibitory.

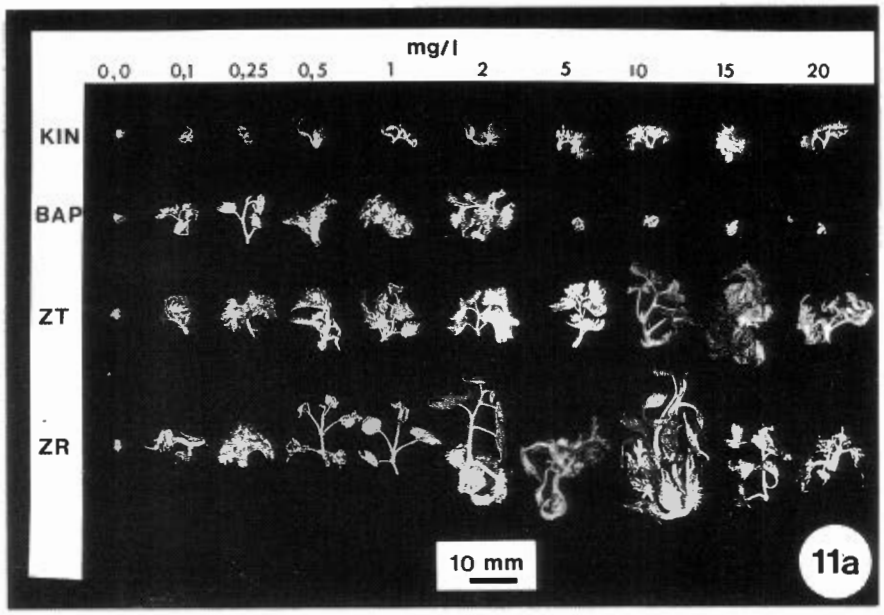
4.3.2.4 Percentages of explants with proliferated shoots

Even at the highest concentration (20 mg/ℓ), kinetin failed to induce axillary shoot growth in all cultures (100%). Both zeatin and ZR induced shoot proliferation in 100% of the cultures at concentrations lower than the optimum levels for shoot elongation, increase in mass and numbers of proliferated shoots. At 2 mg/ℓ, BAP resulted in 100% shoot proliferation for 1-9, but not for Chenin.

4.3.2.5 Effects of optimum levels of different cytokinins

The results are presented in Table 2.

Significantly better shoot elongation as well as increases in mass resulted with ZR at 10 mg/ℓ (optimum level) than with either zeatin, BAP and kinetin (applied at their respective optimum levels of 15, 2 and 20 mg/ℓ). Although highest numbers of proliferated shoots occurred with 10 mg/ℓ ZR, the results did not differ significantly from those recorded for optimum levels of zeatin and BAP. Significantly less proliferated shoots resulted with kinetin at 20 mg/ℓ than with each of the other cytokinins at their optimum levels. With the exception of kinetin - which gave the poorest results - the various cytokinins did not differ significantly in their ability to stimulate axillary shoot growth in cultures.



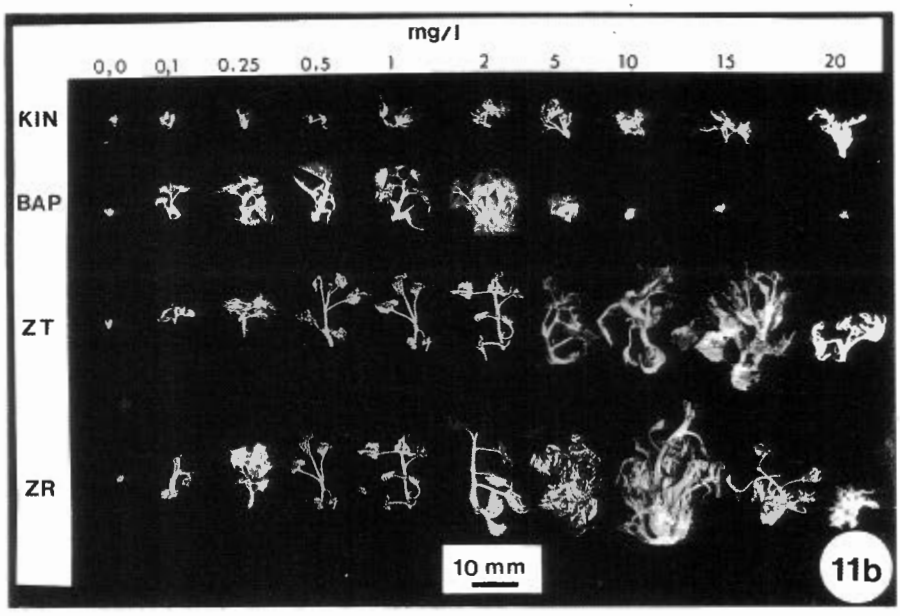


Fig. 11 - In vitro growth responses of excised shoot apices of Chenin (a) and 1-9 (b) to different concentrations of kinetin (KIN), 6-benzylaminopurine (BAP), zeatin (ZT) and zeatin riboside (ZR)

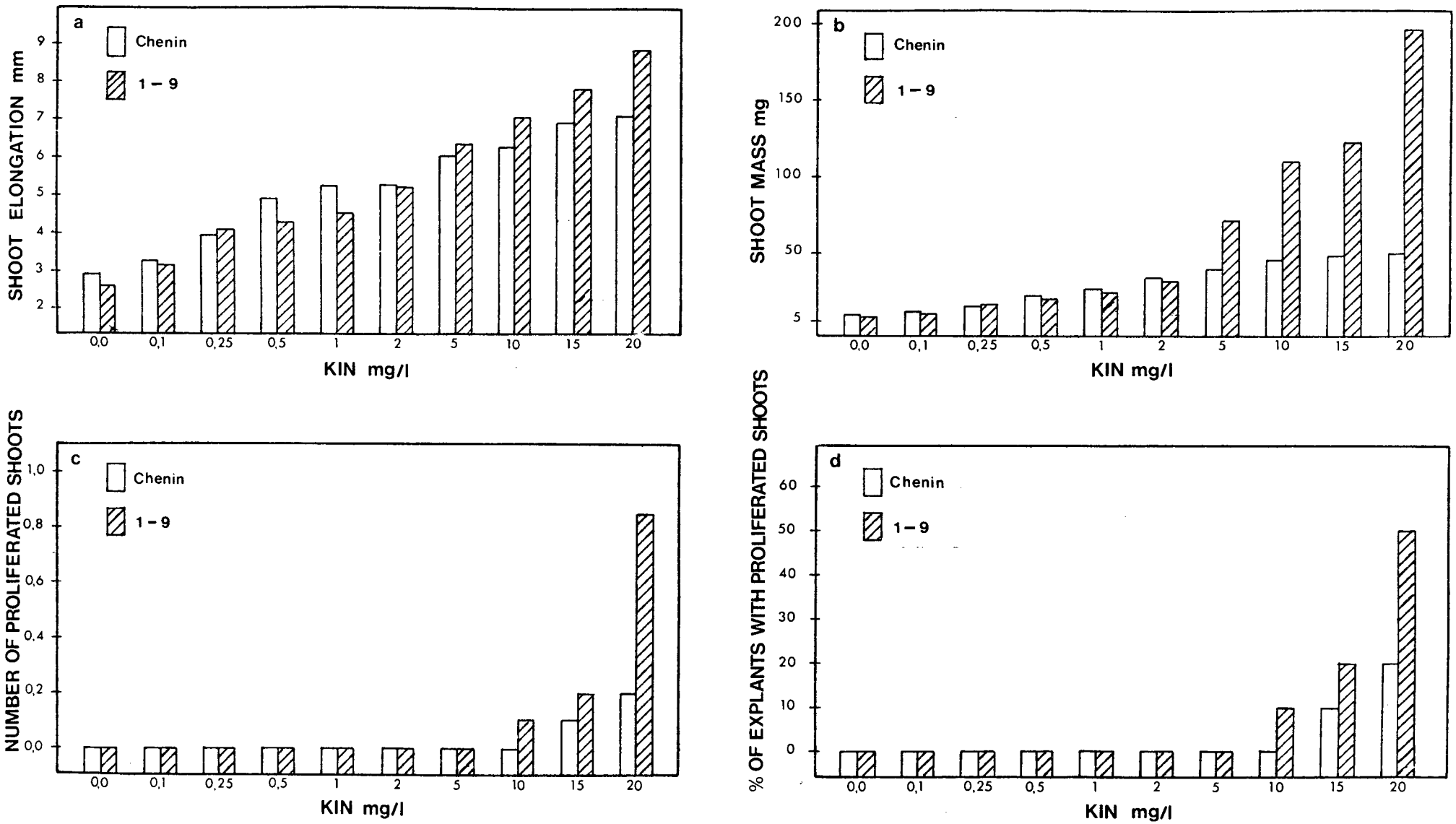


Fig. 12 - The effect of increasing concentrations of kinetin on shoot elongation (a), total shool mass (b), number of proliferated shoots (c), and percentages of explants with proliferated shoots (d), derived from shoot apices of Chenin and 1-9 cultured in vitro

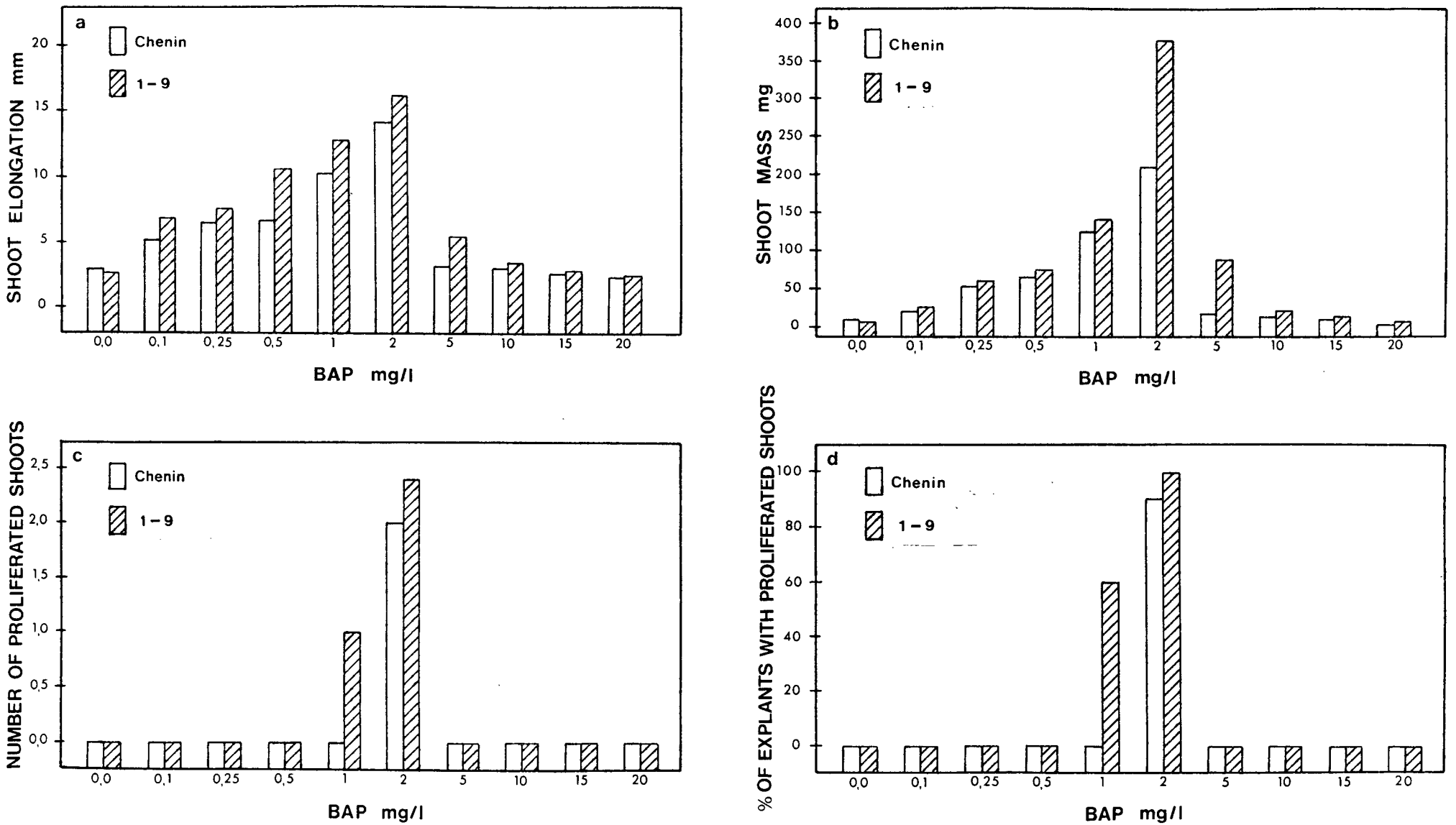


Fig. 13 - The effect of increasing concentrations of 6-benzylaminopurine (BAP) on shoot elongation (a), total shoot mass (b), number of proliferated shoots (c), and percentages of explants with proliferated shoots (d), derived from shoot apices of Chenin and 1-9 cultured in vitro

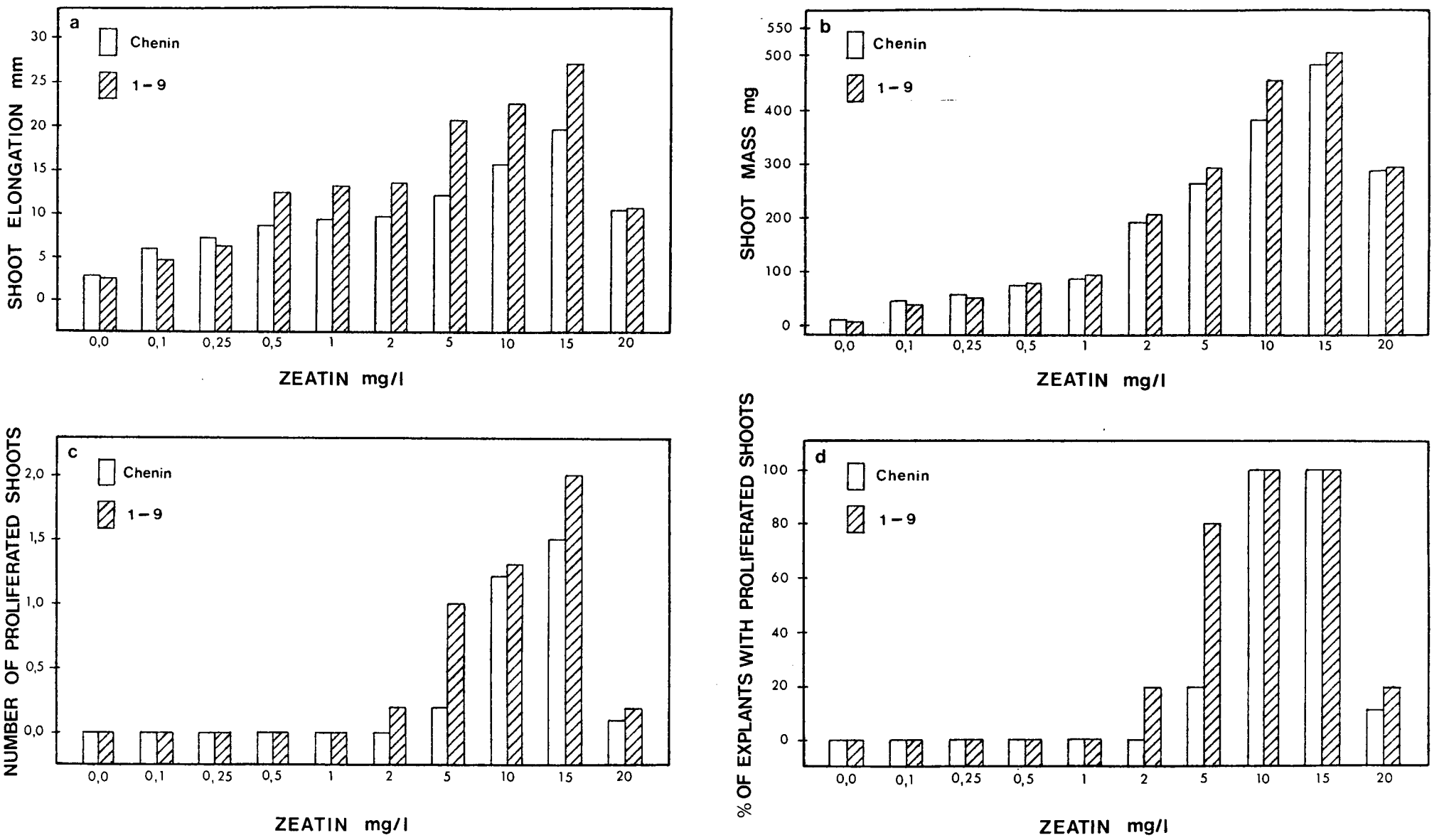


Fig. 14 - The effect of increasing concentrations of zeatin on shoot elongation (a), total shoot mass (b), number of proliferated shoots (c), and percentages of explants with proliferated shoots (d), derived from shoot apices of Chenin and 1-9 cultured in vitro

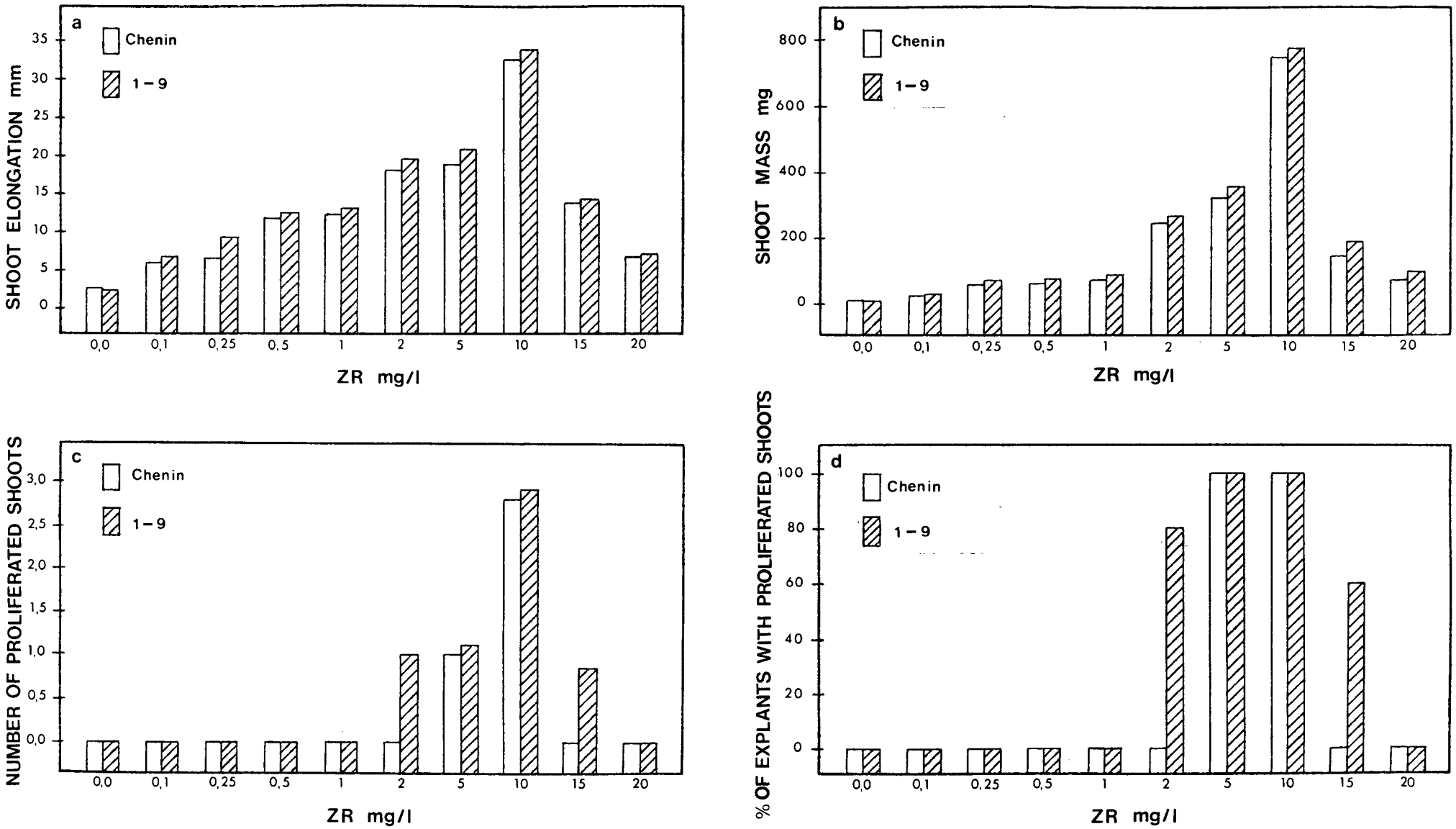


Fig. 15 - The effect of increasing concentrations of zeatin riboside (ZR) on shoot elongation (a), total shoot mass (b), number of proliferated shoots (c), and percentages of explants with proliferated shoots (d), derived from shoot apices of Chenin and 1-9 cultured in vitro

Table 2 - Effects of kinetin, 6-benzylaminopurine (BAP), zeatin and zeatin riboside (ZR) at their optimal levels of 20, 2, 15 and 10 mg/ℓ respectively on shoot elongation, total shoot mass, number of proliferated shoots and percentages of explants with proliferated shoots derived from shoot apices of (1) Chenin and (2) 1-9 cultured in vitro

Treatment		Elongation (mm)		Shoot mass (mg)		Number of proliferated shoots		% explants with proliferated shoots	
Cytokinin	mg/ℓ	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Kinetin	20	7,10 d	8,80 d	49,92 d	195,67 d	0,20 b	0,80 b	20 b	50 b
BAP	2	14,09 c	16,28 c	210,00 c	381,87 c	2,00 a	2,40 a	90 a	100 a
Zeatin	15	19,90 b	26,90 b	489,55 b	502,35 b	1,50 a	2,00 a	100 a	100 a
ZR	10	32,60 a	33,72 a	749,32 a	785,68 a	2,80 a	2,90 a	100 a	100 a

a - d: Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level of significance.

4.4 Effects of BAP and ZR alone and combined with NAA

4.4.1 Objective and procedure

Detached shoot apices of woody plant species (apple, cherry, pear and plum) responded with enhanced shoot production to combinations of BAP and NAA at appropriate levels (Cheng, 1978, 1979). Although the potential of BAP:NAA combinations for induction of shoot proliferation of excised grapevine shoot apices has been indicated (Chée and Pool, 1982), detailed data are lacking on optimal levels of BAP:NAA ratios. This experiment was conducted to determine responses of excised shoot apices of Chenin and 1-9 to different concentrations of BAP and ZR alone and in combination with NAA.

The standard procedure (3.1, 3.2, 3.3) was followed and the treatments listed in Figs. 16 and 17 were applied. Each treatment consisted of 10 explants replicated twice; the culture period was 42 days. Data for each cultivar were subjected to analysis of variance followed by the Student Newmann-Keuls plural comparison test (Snedecor and Cochran, 1967).

4.4.2 Results

The results are presented in Figs. 16, 17, 18, 19, 20 and 21, and in Appendices 5.1, 5.2, 6.1 and 6.2. Unless otherwise indicated, results refer to both cultivars.

4.4.2.1 Shoot elongation

Singly, BAP and ZR induced maximum elongation of primary shoots at 2 and 10 mg/ℓ respectively. In the presence of 3,5 mg/ℓ BAP

elongation was suppressed (significantly lower than levels obtained with 1 - 2 mg/ℓ for Chenin and 0,5 - 2 mg/ℓ for 1-9). In the absence of cytokinin, elongation was not significantly suppressed by NAA.

Combinations of BAP (0,5 - 3,5 mg/ℓ) and ZR (1 - 10 mg/ℓ) with 0,1 mg/ℓ NAA stimulated shoot elongation as compared to control treatments (no cytokinin). However, elongation levels induced with applications of the cytokinins singly were not attained by any combination.

4.4.2.2 Shoot mass

With one exception (2 mg/ℓ BAP plus 0,1 mg/ℓ NAA in 1-9), the increase in shoot mass obtained with 2 mg/ℓ BAP was significantly higher if compared to all other treatments.

Combination of ZR (10 mg/ℓ) with NAA (0,1 mg/ℓ) gave rise to a significantly higher shoot mass than that obtained with 10 mg/ℓ ZR alone (Chenin). This combination was the most effective of all treatments (Chenin). In general, higher masses resulted from applications of either BAP (0,5 - 3,5 mg/ℓ) or ZR (1 - 10 mg/ℓ) singly, if compared with combinations of BAP or ZR with the different NAA concentrations (1-9). These differences, however, were not always statistically significant.

4.4.2.3 Callus growth at basal cut surfaces of explants

No callus growth occurred in the presence of BAP and ZR applied singly. In the absence of cytokinin, slight but not significant callus growth occurred with NAA at levels of 1 mg/ℓ and above.

Low concentrations of NAA (0,1 mg/ℓ) resulted in callus growth only when applied in combination with ZR (1 - 10 mg/ℓ); the increase was not significant, however.

In the presence of 0,5 to 2 mg/ℓ BAP, callus masses increased with increasing concentrations of NAA. Maximum callus resulted with 1 mg/ℓ BAP and 5 mg/ℓ NAA for Chenin and with 2 mg/ℓ BAP and 2 mg/ℓ NAA for 1-9.

Application of ZR (1 - 10 mg/ℓ) in combination with NAA (0,1 - 5 mg/ℓ) resulted in an almost linear increase in callus growth (Chenin). Maximum callus masses (significant at the 5% level) were induced with the highest levels of ZR and NAA in combination (10 and 5 mg/ℓ respectively) (Chenin). A different response was recorded for 1-9: Significantly better callus growth occurred at lower levels of both NAA and ZR in combination (2 mg/ℓ with 1 mg/ℓ and 1 - 2 mg/ℓ with 2 - 5 mg/ℓ respectively). A significant suppression followed at higher NAA levels in combination with ZR (5 mg/ℓ with 1 - 5 mg/ℓ). Callus growth was also markedly suppressed in combinations of 10 mg/ℓ ZR with 1 and 2 mg/ℓ NAA.

4.4.2.4 Shoot proliferation

No shoot proliferation occurred in the absence of cytokinin. In the presence of BAP and ZR, alone or with 0,1 mg/ℓ NAA, axillary shoot growth proceeded. Highest numbers of proliferated shoots resulted with 2 mg/ℓ BAP and 10 mg/ℓ ZR. A complete inhibition occurred with BAP at higher levels (3,5 mg/ℓ).

Although axillary shoot growth occurred with certain BAP:NAA combinations, highest proliferation rates (not always statistically significant) were induced by BAP alone at 2 mg/ℓ. Combined applications of 1 - 2 mg/ℓ ZR with NAA were ineffective for shoot proliferation. However, proliferation rates increased in response to combinations of ZR at higher levels (5 and 10 mg/ℓ) with 0,1 mg/ℓ NAA. Shoot numbers recorded for the above combinations were significantly better than those for ZR alone (5 and 10 mg/ℓ) (Chenin).

Inhibitory effects of NAA at higher levels (1 - 5 mg/ℓ) could not be alleviated by adding either BAP (0,5 - 3,5 mg/ℓ) or ZR (1 - 10 mg/ℓ).

4.4.2.5 Percentages of explants with proliferated shoots

Treatments comprising 2 mg/ℓ BAP alone and in combination with 0,1 mg/ℓ NAA gave rise to shoot proliferation in all cultures (100%) (1-9). In Chenin none of the treatments induced shoot proliferation in 100% of the cultures.

Single applications of ZR (5 and 10 mg/ℓ) resulted in 100% shoot proliferation. Although more shoots proliferated with 5 mg/ℓ ZR combined with 0,1 mg/ℓ NAA than with 5 mg/ℓ ZR alone, the above combination failed to induce proliferation in all cultures (100%).

4.4.2.6 Root formation

Rooting occurred only in Chenin at appropriate levels of ZR and

NAA in combination. The results are presented in Fig. 17a and Table 3.

No rooting occurred within the culture period of 42 days in the presence of ZR or NAA alone as well as in combinations including the above growth substances at their highest levels.

Although a combination of 2 mg/ℓ ZR with 1 mg/ℓ NAA proved the most effective for root initiation (significant at the 5% level), root elongation was markedly suppressed as compared to 2 mg/ℓ ZR plus 0,1 mg/ℓ NAA. Root elongation was stimulated with increasing concentrations of ZR (up to 5 mg/ℓ) in combination with 0,1 and 1 mg/ℓ NAA respectively. Maximum elongation was recorded with 5 mg/ℓ ZR and 0,1 mg/ℓ NAA. However, in combination with higher NAA levels (2 mg/ℓ), root elongation became suppressed with increasing concentrations of ZR.

The highest root mass resulted from 2 mg/ℓ ZR in combination with 0,1 mg/ℓ NAA, although the root number was less than that recorded for the optimum combination (2 mg/ℓ ZR and 1 mg/ℓ NAA). Apart from 5 mg/ℓ ZR in combination with 2 mg/ℓ NAA, all cultures that developed roots showed 100% rooting.

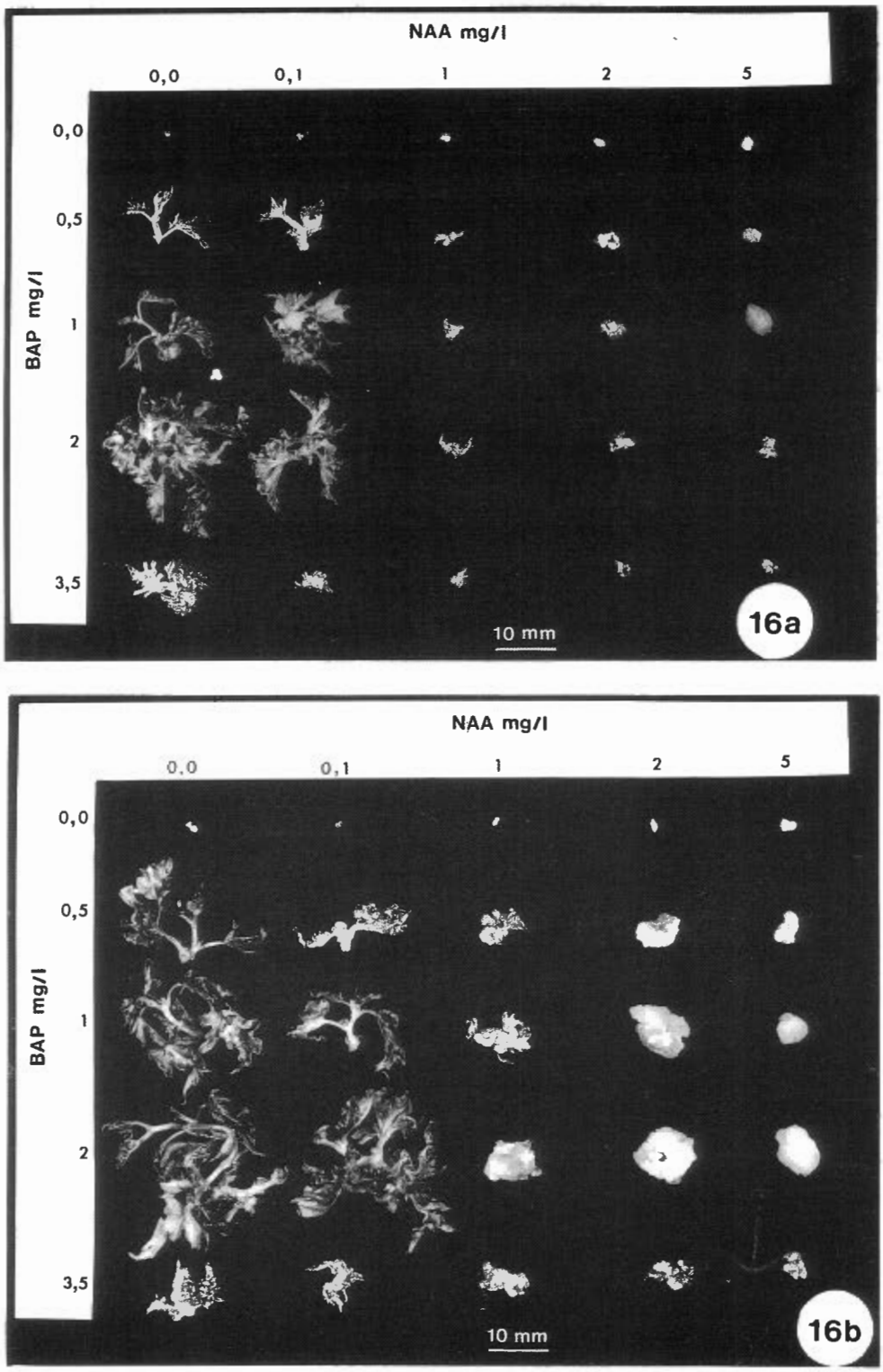


Fig. 16 - In vitro growth responses of excised shoot apices of Chenin (a) and 1-9 (b) to different concentration ratios of 6-benzylaminopurine (BAP) and 1-naphthaleneacetic acid (NAA)

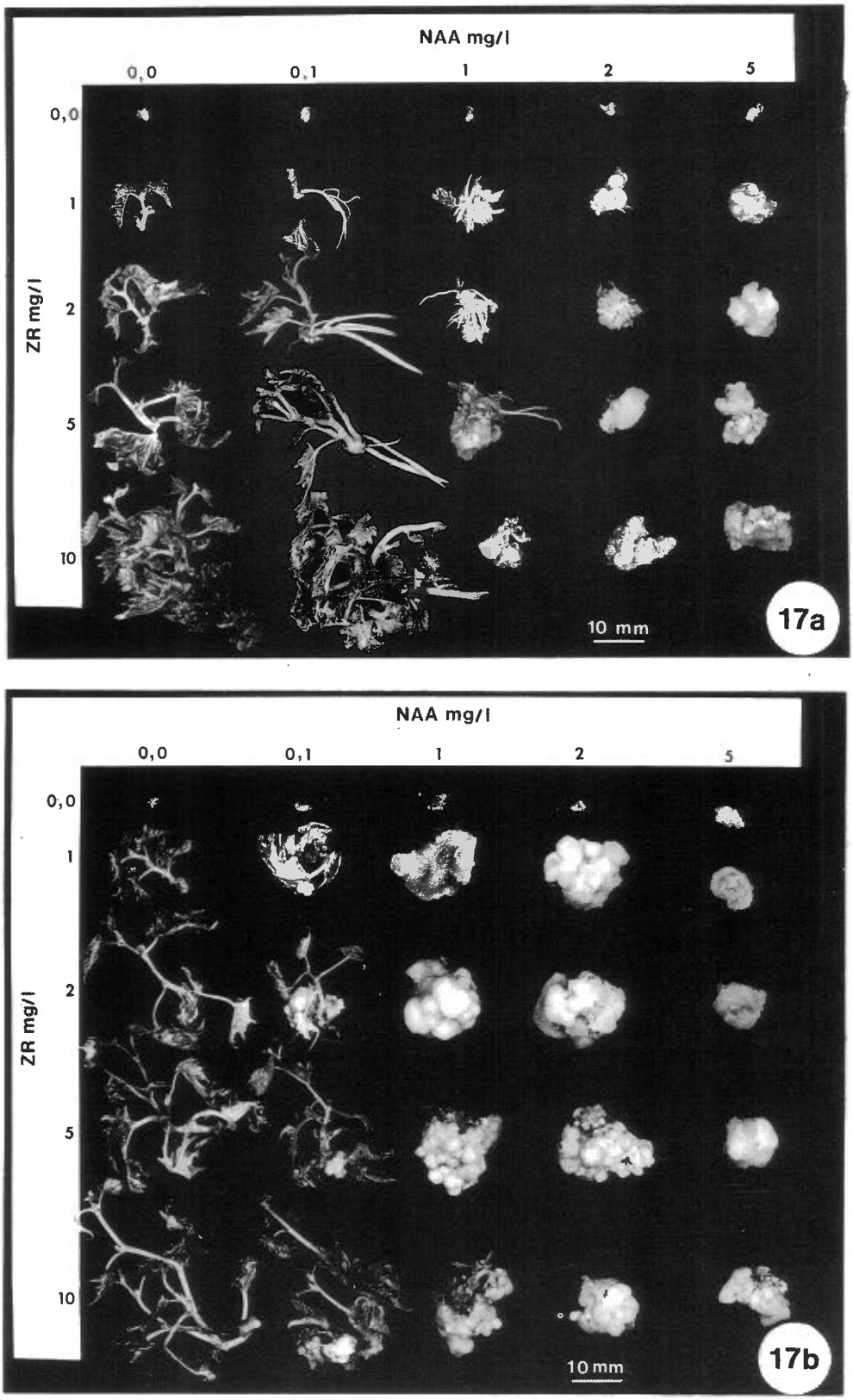


Fig. 17 - In vitro growth responses of excised shoot apices of Chenin (a) and 1-9 (b) to different concentration ratios of zeatin riboside (ZR) and 1-naphthaleneacetic acid (NAA)

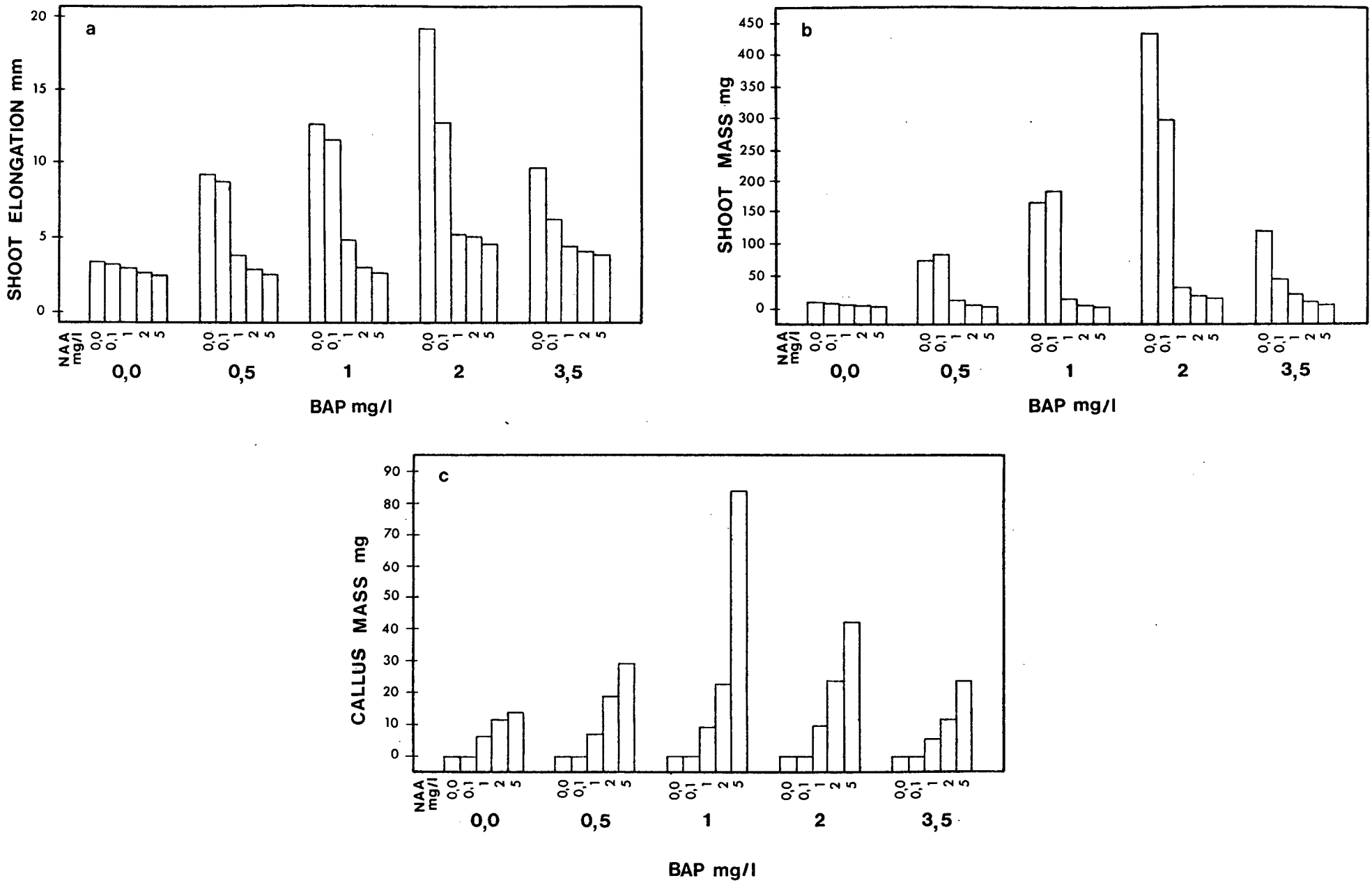


Fig. 18 (a-c) - The effect of different concentration ratios of 6-benzylaminopurine (BAP) and 1-naphthaleneacetic acid (NAA) on shoot elongation (a), total shoot mass (b), and callus mass (c), derived from shoot apices of Chenin cultured in vitro

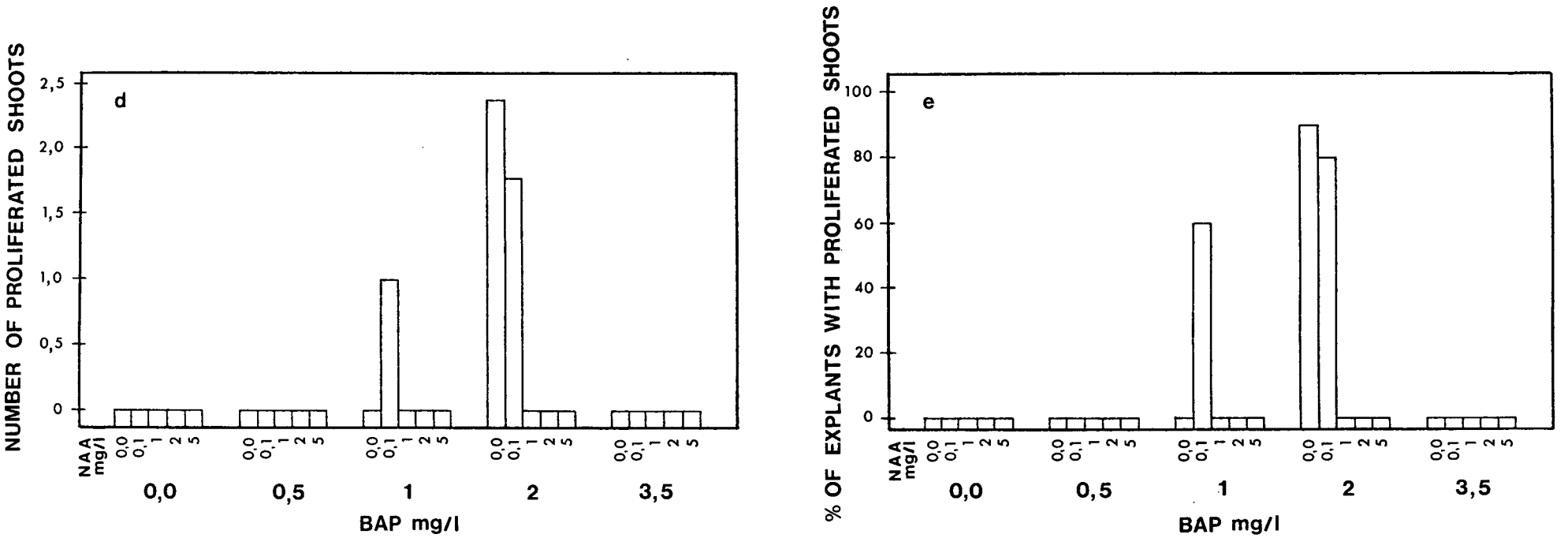


Fig. 18 (d-e) - The effect of different concentration ratios of 6-benzylaminopurine (BAP) and 1-naphthaleneacetic acid (NAA) on number of proliferated shoots (d), and percentages of explants (e), derived from shoot apices of Chenin cultured in vitro

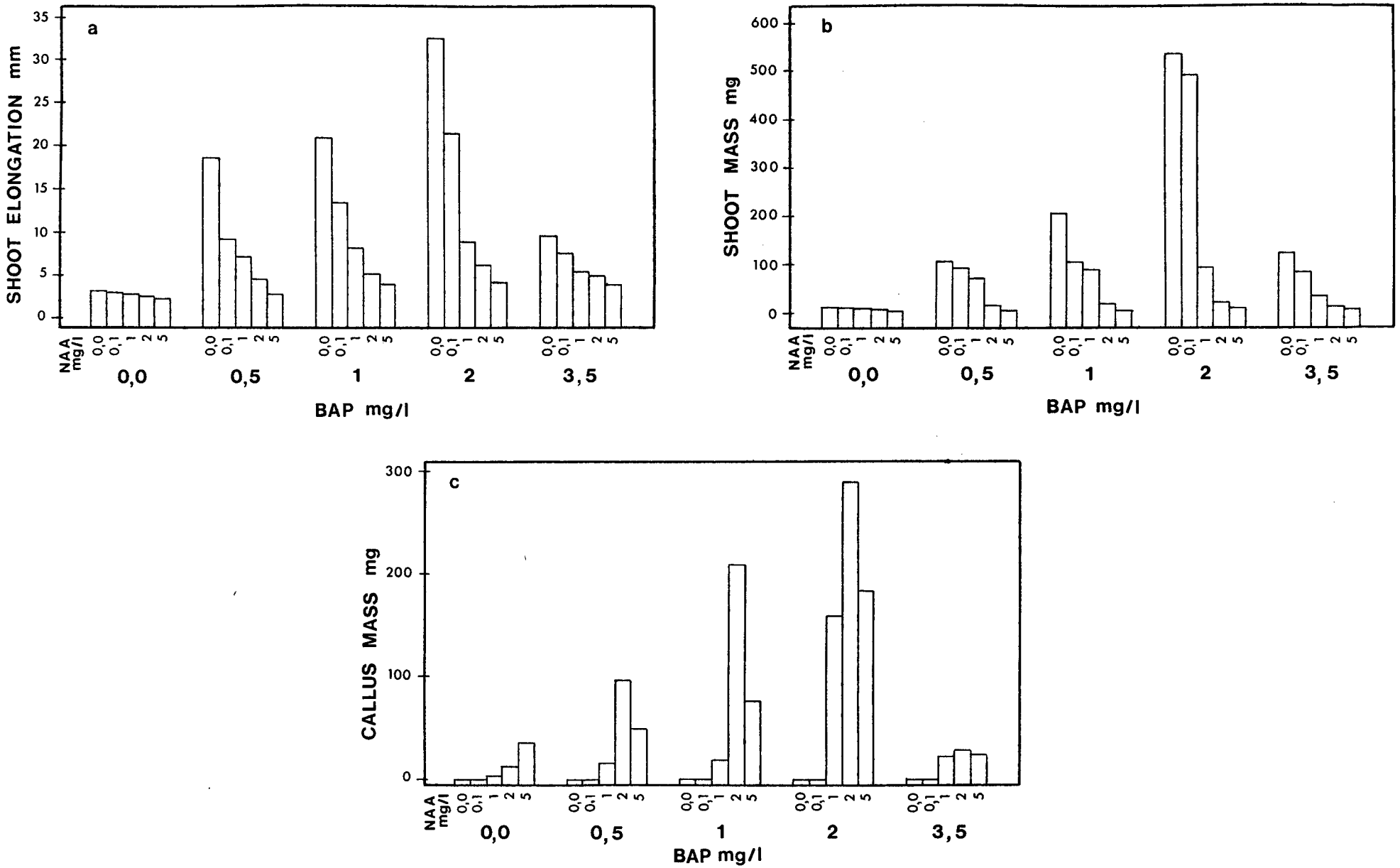


Fig. 19 (a-c) - The effect of different concentration ratios of 6-benzylaminopurine (BAP) and 1-naphthaleneacetic acid (NAA) on shoot elongation (a), total shoot mass (b), and callus mass (c), derived from shoot apices of 1-9 cultured in vitro

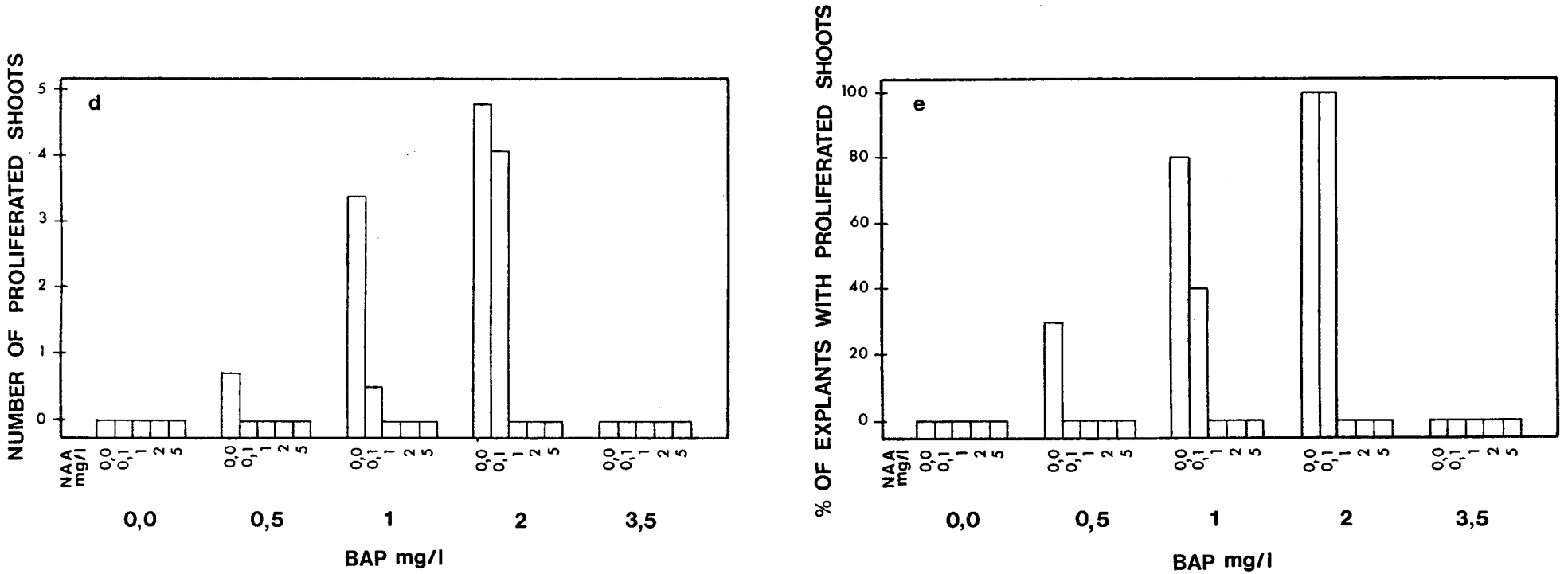


Fig. 19 (d-e) - The effect of different concentration ratios of 6-benzylaminopurine (BAP) and 1-naphthaleneacetic acid (NAA) on number of proliferated shoots (d), and percentages of explants with proliferated shoots (e), derived from shoot apices of 1-9 cultured in vitro

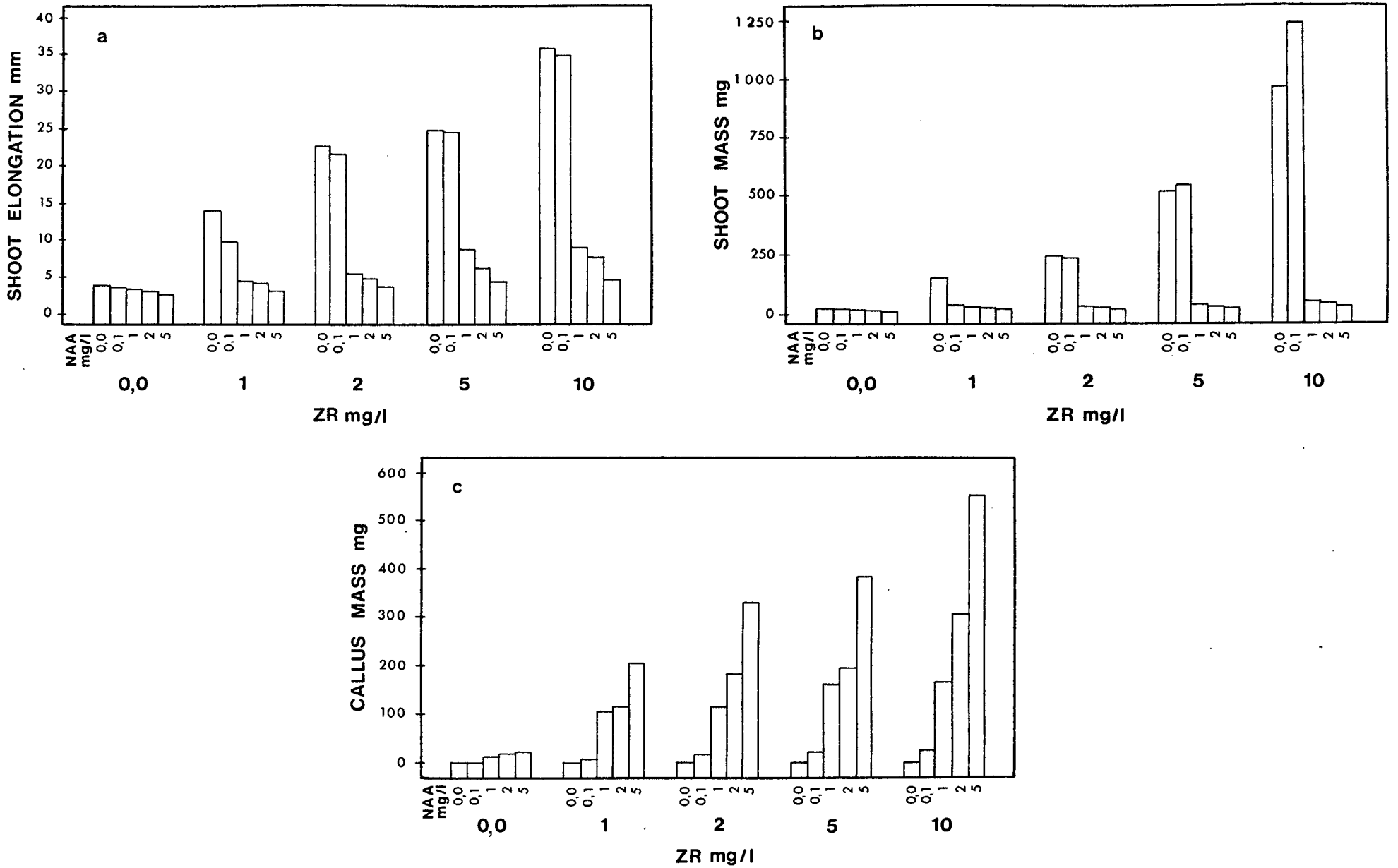


Fig. 20 (a-c) - The effect of different concentration ratios of zeatin riboside (ZR) and 1-naphthaleneacetic acid (NAA) on shoot elongation (a), total shoot mass (b), and callus mass (c), derived from shoot apices of Chenin cultured in vitro

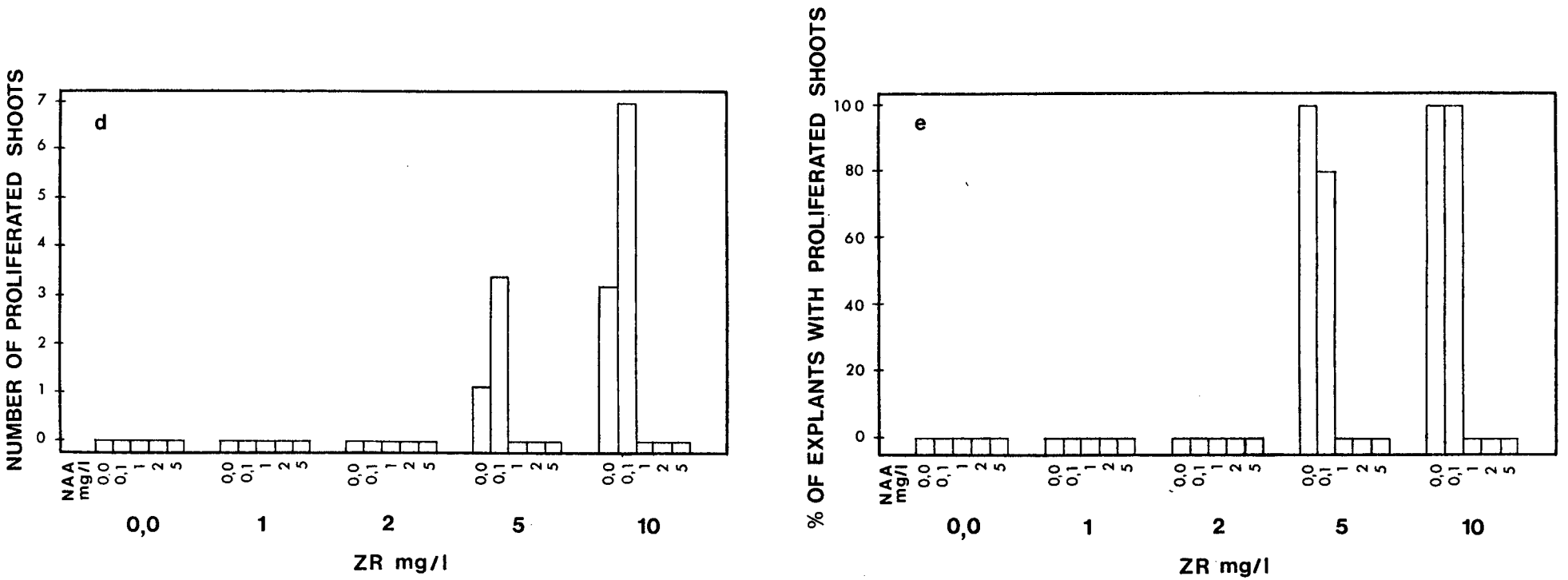


Fig. 20 (d-e) - The effect of different concentration ratios of zeatin riboside (ZR) and 1-naphthalene-acetic acid (NAA) on number of proliferated shoots (d), and percentages of explants with proliferated shoots (e), derived from shoot apices of Chenin cultured in vitro

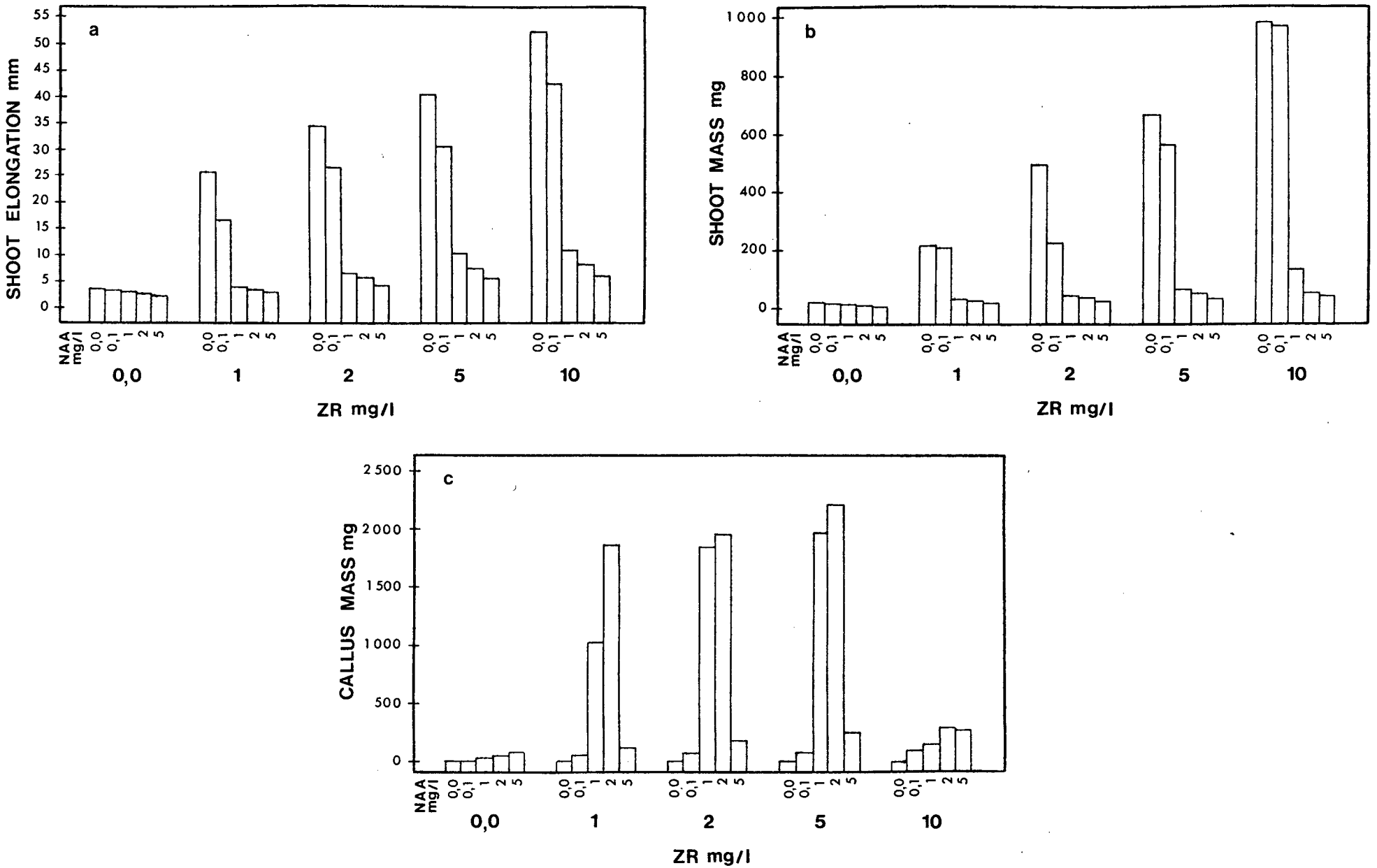


Fig. 21 (a-c) - The effect of different concentration ratios of zeatin riboside (ZR) and 1-naphthalene-acetic acid (NAA) on shoot elongation (a), total shoot mass (b), and callus mass (c), derived from shoot apices of 1-9 cultured in vitro

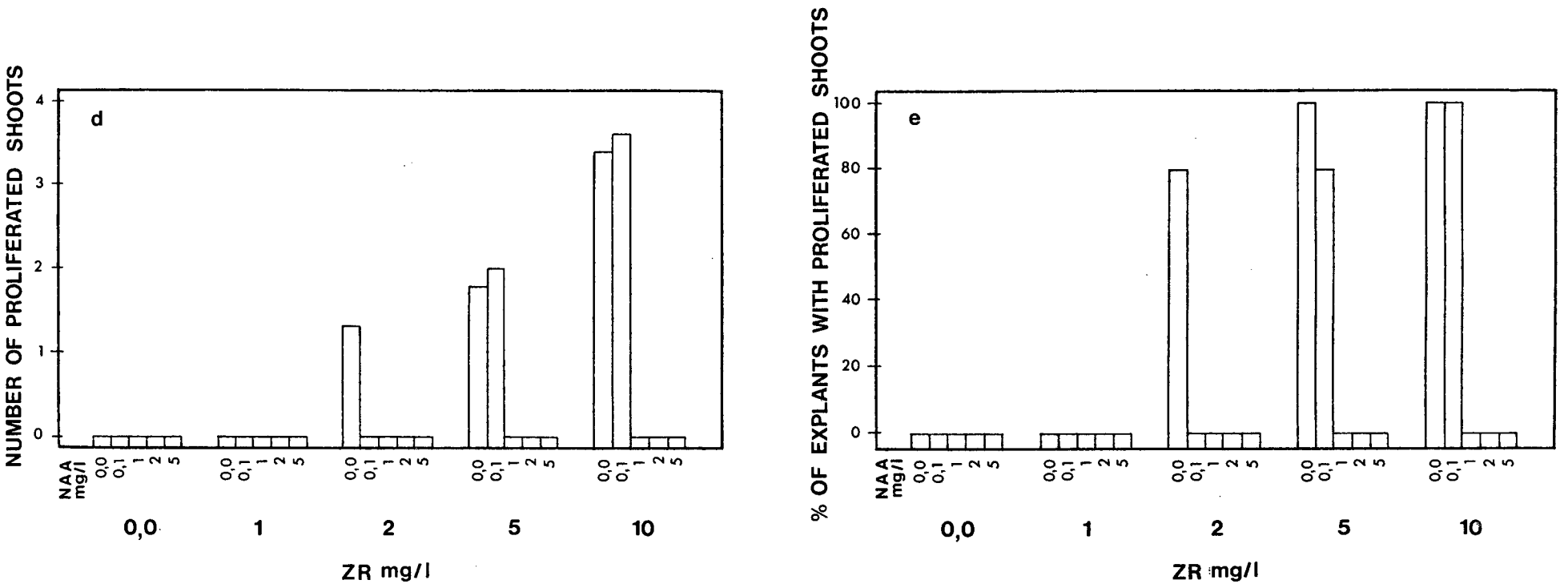


Fig. 21 (d-e) - The effect of different concentration ratios of zeatin riboside (ZR) and 1-naphthalene-acetic acid (NAA) on number of proliferated shoots (d), and percentages of explants with proliferated shoots (e), derived from shoot apices of 1-9 cultured in vitro

Table 3 - The effect of different concentration ratios of zeatin riboside (ZR) and 1-naphthaleneacetic acid (NAA) on root initiation, root elongation, total root mass and percentages of explants with root formation derived from shoot apices of Chenin blanc cultured in vitro

Treatment (mg/ℓ) ZR : NAA		Number of roots	Root elongation (mm)	Root mass (mg)	% of explants with root formation
0,0	0,0	0,00 g	0,00 i	0,00 h	0 c
0,0	0,1	0,00 g	0,00 i	0,00 h	0 c
0,0	1,0	0,00 g	0,00 i	0,00 h	0 c
0,0	2,0	0,00 g	0,00 i	0,00 h	0 c
0,0	5,0	0,00 g	0,00 i	0,00 h	0 c
1,0	0,0	0,00 g	0,00 i	0,00 h	0 c
1,0	0,1	2,60 e	15,50 c	10,28 f	100 a
1,0	1,0	5,50 b	6,60 f	28,28 d	100 a
1,0	2,0	2,10 e	4,60 g	12,18 f	100 a
1,0	5,0	0,00 g	0,00 i	0,00 h	0 c
2,0	0,0	0,00 g	0,00 i	0,00 h	0 c
2,0	0,1	5,50 b	18,90 b	54,91 a	100 a
2,0	1,0	6,20 a	9,80 e	37,82 c	100 a
2,0	2,0	2,50 e	4,20 g	12,19 f	100 a
2,0	5,0	0,00 g	0,00 i	0,00 h	0 c
5,0	0,0	0,00 g	0,00 i	0,00 h	0 c
5,0	0,1	4,70 c	21,10 a	48,49 b	100 a
5,0	1,0	3,10 d	11,90 d	18,39 e	100 a
5,0	2,0	1,00 f	1,80 h	6,25 g	50 b
5,0	5,0	0,00 g	0,00 i	0,00 h	0 c
10,0	0,0	0,00 g	0,00 i	0,00 h	0 c
10,0	0,1	0,00 g	0,00 i	0,00 h	0 c
10,0	1,0	0,00 g	0,00 i	0,00 h	0 c
10,0	2,0	0,00 g	0,00 i	0,00 h	0 c
10,0	5,0	0,00 g	0,00 i	0,00 h	0 c

a - i: Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level.

4.5 Effects of ZR alone and in combination with GA_3

4.5.1 Objective and procedure

Irrespective of stimulatory effects on primary shoot elongation (Morel, 1964a, b), gibberellic acid (GA_3) has found limited application in shoot apex cultures, due to suppressing effects on callus and root initiation and axillary shoot growth (Murashige, 1977; Hussey, 1980; Wang and Hu, 1980; Allan, 1981a,b). The question arose as to whether inhibitory effects of GA_3 on axillary shoot growth from excised grapevine shoot apices could be overcome by the addition of cytokinins. In this experiment responses of detached shoot apices of Chenin and 1-9 to combined applications of ZR and GA_3 were investigated.

The procedure as outlined earlier (3.1, 3.2, 3.3) was followed and the explants were subjected to treatments indicated in Fig. 22. Each treatment consisted of two replicates of 10 explants each. Due to browning and deterioration of cultures over prolonged periods the experiment was terminated after 18 days. Data for each cultivar were subjected to analysis of variance followed by the Student Newmann-Keuls plural comparison test (Snedecor and Cochran, 1967).

4.5.2 Results

The results are presented in Figs. 22, 23 and 24, and in Appendices 7.1 and 7.2. Unless otherwise indicated results are related to both cultivars.

4.5.2.1 Shoot elongation

Primary shoot elongation was stimulated in the presence of either ZR or GA_3, applied singly. Maximum elongation resulted at the highest levels (10 mg/ℓ) of each of the growth substances tested. However, if compared with control treatments, mean shoot length increased significantly only for 5 - 10 mg/ℓ ZR.

Combined applications of ZR and GA_3 had a favourable effect on shoot elongation. Highest levels resulted with 10 mg/ℓ ZR and 10 mg/ℓ GA_3 (significantly higher than that recorded for ZR and GA_3 applied singly at 10 mg/ℓ) (Chenin). In 1-9 lower levels of ZR (5 mg/ℓ) in combination with 10 mg/ℓ GA_3 accounted for maximum elongation (significantly higher than that recorded for 10 mg/ℓ ZR and 10 mg/ℓ GA_3).

4.5.2.2 Shoot mass

Shoot mass increased in response to increasing concentrations of ZR and GA_3 applied singly, reaching a maximum at the highest levels tested (10 mg/ℓ). However, GA_3 alone contributed only to a limited extent to the mass increase. After 18 days mass increase was not significantly better than that recorded for control treatments.

Maximum increase in mass resulted with combined applications of 10 and 5 mg/ℓ ZR with 10 mg/ℓ GA_3 (Chenin and 1-9 respectively).

4.5.2.3 Leaf expansion

In the presence of ZR alone the number of expanded leaves in-

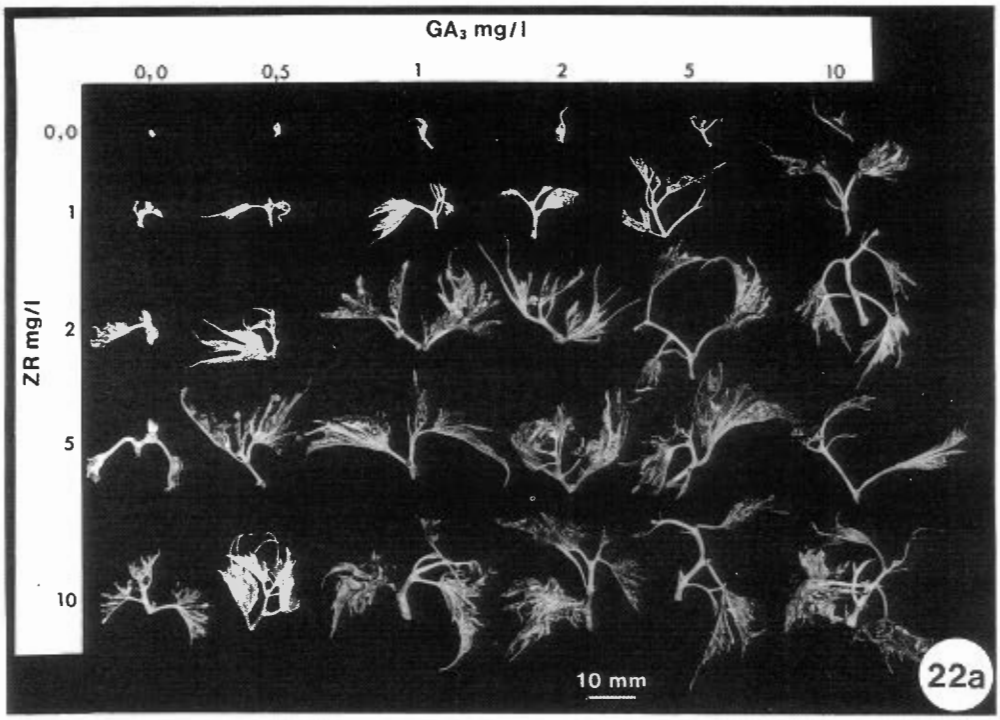
creased with increasing concentrations of ZR, reaching a maximum at 10 mg/ℓ. Although leaf expansion occurred in the absence of ZR with 5 and 10 mg/ℓ GA_3, only a few leaves were expanded.

Combined applications of the growth substances tested induced more expanded leaves than individual treatments. A combination of 10 mg/ℓ ZR and 10 mg/ℓ GA_3 proved the most effective in Chenin. In 1-9 highest leaf numbers resulted at lower GA_3 concentrations (0,5 and 1 mg/ℓ) in combination with 10 mg/ℓ ZR.

It was also noted - not shown in the results - that explants responded with tendril formation to GA_3 singly (2 - 10 mg/ℓ) as well as to combinations of 1 - 10 mg/ℓ ZR with 0,5 - 10 mg/ℓ GA_3. In the presence of ZR alone no tendrils formed.

4.5.2.4 Percentages of explants with expanded leaves

Only those explants treated with the highest concentrations of ZR alone (10 mg/ℓ for Chenin and 5 and 10 mg/ℓ for 1-9) gave rise to leaf expansion in 100% of the cultures, regardless of the GA_3 level. GA_3 by itself failed to induce leaf expansion in all cultures (100%). Leaf expansion could be induced in 100% of the cultures at lower concentrations of ZR when applied in combination with higher GA_3 levels.



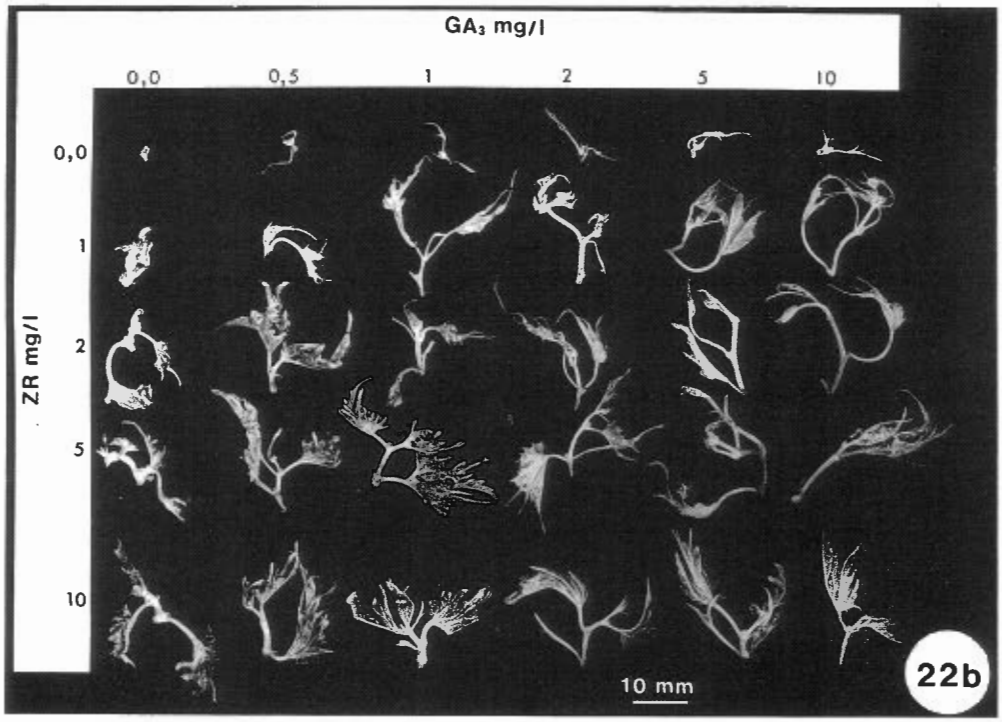


Fig. 22 - In vitro growth responses of excised shoot apices of Chenin (a) and 1-9 (b) to different concentration ratios of zeatin riboside (ZR) and gibberellic acid (GA_3)

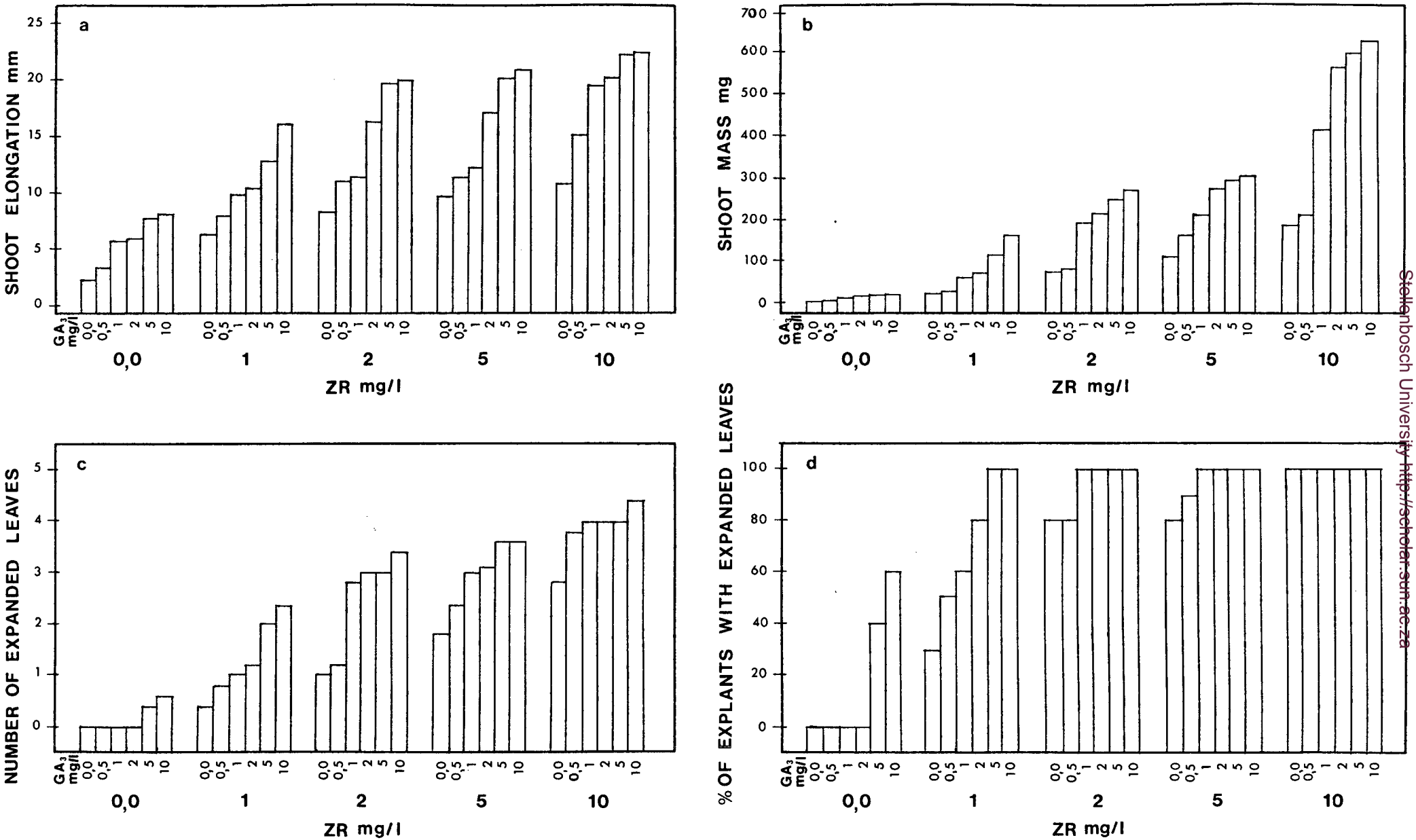


Fig. 23 - The effect of different concentration ratios of zeatin riboside (ZR) and gibberellic acid (GA_3) on shoot elongation (a), total shoot mass (b), number of expanded leaves (c), and percentages of explants with expanded leaves (d), derived from shoot apices of Chenin cultured in vitro

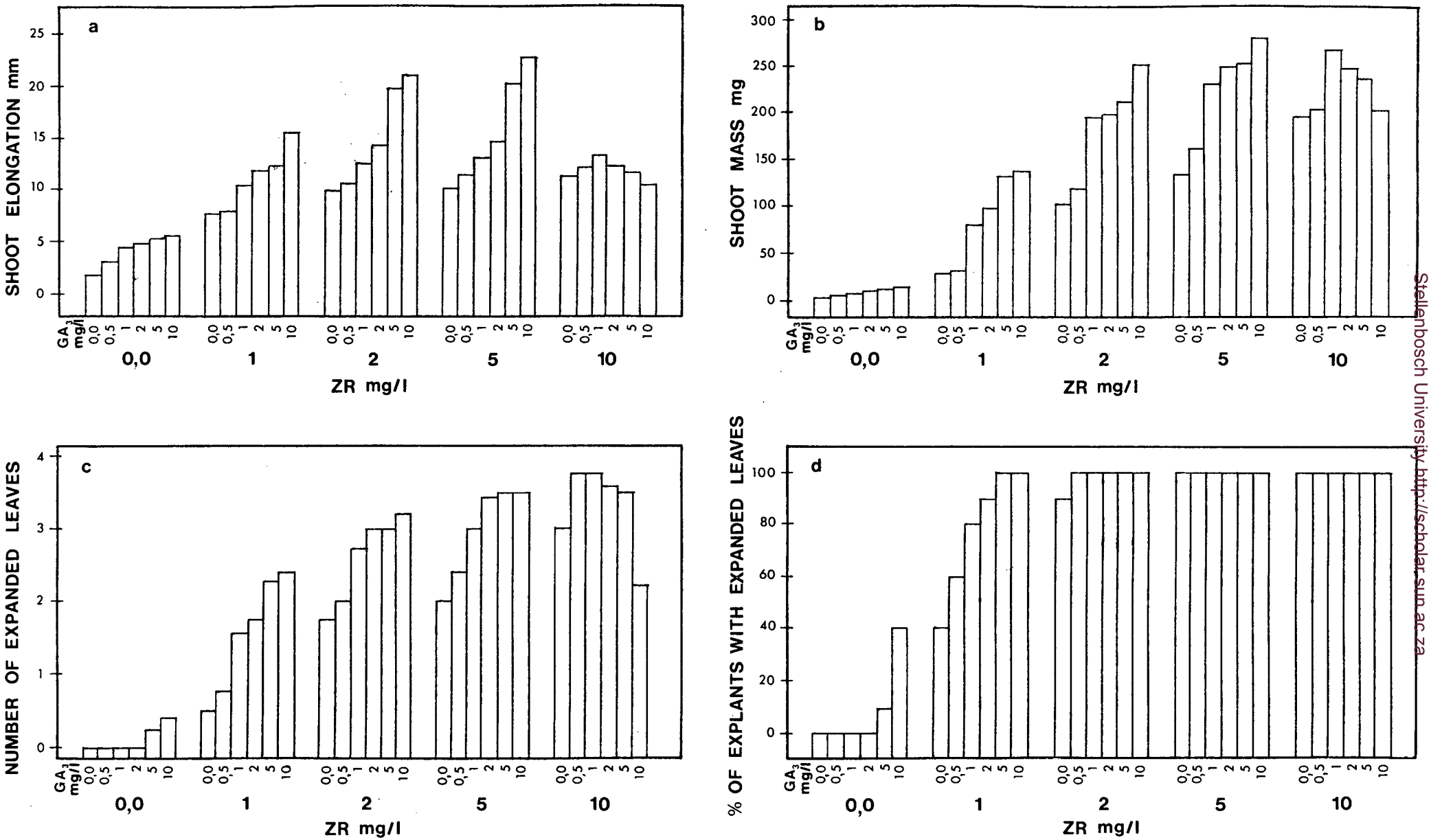


Fig. 24 - The effect of different concentration ratios of zeatin riboside (ZR) and gibberellic acid (GA_3) on shoot elongation (a), total shoot mass (b), number of expanded leaves (c), and percentages of explants with expanded leaves (d), derived from shoot apices of 1-9 cultured in vitro

4.6 Effects of combined applications of BAP and ZR

4.6.1 Objective and procedure

Although for a variety of woody plants it has been shown that detached shoot apices cultured *in vitro* responded with enhanced axillary shoot growth in the presence of cytokinin, whether alone or in combination with auxin (Abbott and Whiteley, 1976; Tabachnik and Kester, 1977; Chaturvedi *et al.*, 1978; Hammerschlag, 1980; Singha, 1980), detailed information is lacking on the effects of combined applications of different cytokinins. This experiment was conducted to investigate responses of excised shoot apices of Chenin to combined applications of BAP and ZR.

The procedure as outlined earlier (3.1, 3.2, 3.3) was followed and explants were subjected to treatments listed in Fig. 25. Each treatment comprised two replicates of 10 explants each, with a culture period of 42 days. Results were subjected to analysis of variance followed by the Student Newmann-Keuls plural comparison test (Snedecor and Cochran, 1967).

4.6.2 Results

The results are presented in Figs. 25, 26, 27 and 28, and in Appendix 8.

4.6.2.1 Shoot elongation

Elongation rates increased considerably in response to ZR treatments alone. Maximum elongation was recorded at 10 mg/ℓ. In the presence of BAP alone, maximum elongation resulted at 2 mg/ℓ;

however, at 5 mg/ℓ it was severely inhibited. Combined applications of BAP (0,5 - 2 mg/ℓ) and ZR (1 - 5 mg/ℓ) stimulated elongation significantly as compared to control treatments. In comparison to elongation with 10 mg/ℓ ZR, inhibition resulted when 0,5 - 2 mg/ℓ BAP was added. There were no significant differences between elongation levels with BAP (5 mg/ℓ) combined with ZR (1 - 10 mg/ℓ) and the control.

4.6.2.2 Shoot mass

Maximum increase in mass was achieved with a combination of BAP and ZR (both at 2 mg/ℓ). Although shoot elongation was inhibited with 5 mg/ℓ BAP, singly and in combination with 1 - 10 mg/ℓ ZR, mass tended to increase slightly; however, not statistically significant from the control.

4.6.2.3 Shoot proliferation

Singly, ZR induced maximum shoot proliferation at the highest concentration tested (10 mg/ℓ). Proliferation rates with optimum levels of ZR (10 mg/ℓ) and BAP (2 mg/ℓ), supplied singly, did not differ significantly. At 5 mg/ℓ, BAP resulted in complete inhibition of shoot proliferation. Shoot proliferation was stimulated by a combination of the two cytokinins, a combination of BAP and ZR (both at 2 mg/ℓ) proving the most effective. It was also evident that the agar medium (Fig. 27) did not inhibit submerged growth, i.e. shoot elongation and proliferation.

4.6.2.4 Percentages of explants with proliferated shoots

Only those explants treated with the highest concentrations

(5 and 10 mg/ℓ) of ZR singly induced shoot proliferation in all cultures (100%). BAP by itself was most effective at 2 mg/ℓ. Shoot proliferation in 100% of the cultures could be achieved with lower concentrations of cytokinins in combination than with each cytokinin applied separately.

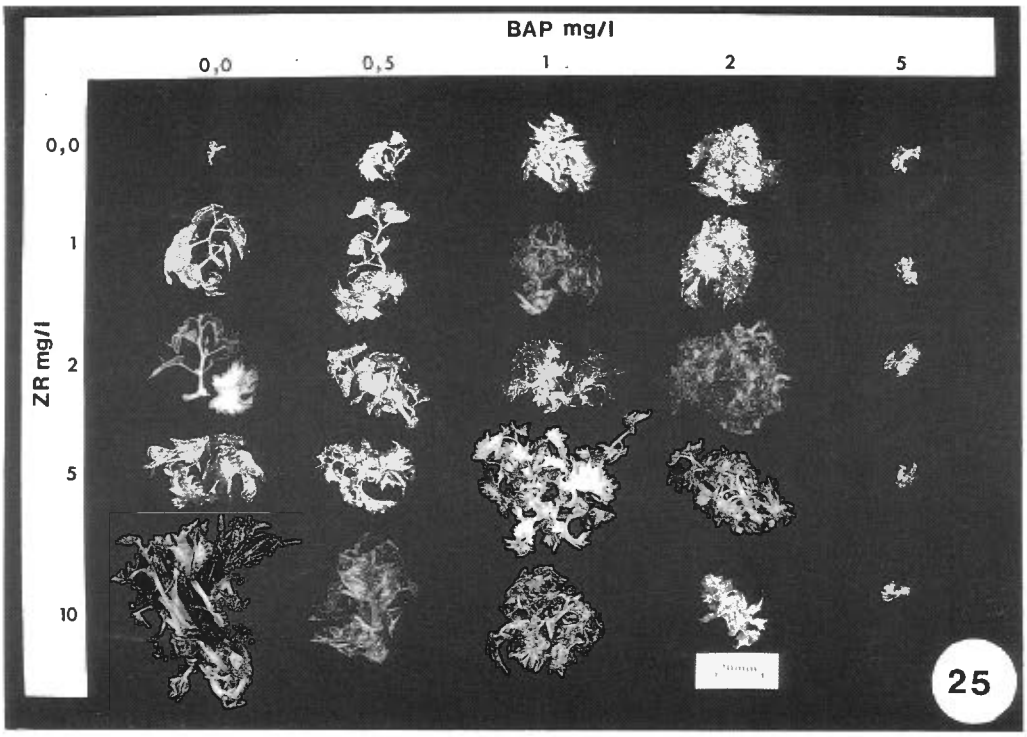
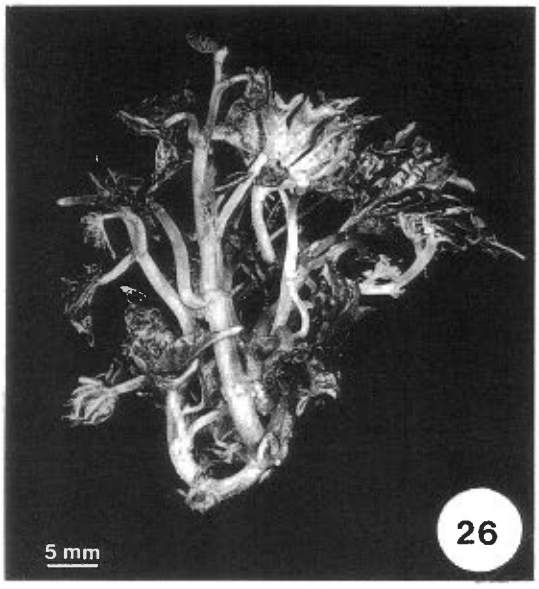


Fig. 25 - In vitro growth responses of excised shoot apices of Chenin to different concentration ratios of 6-benzylaminopurine (BAP) and zeatin riboside (ZR)



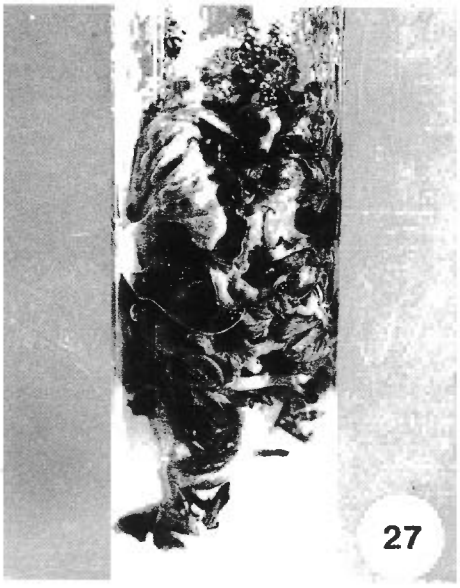


Fig. 26 - Shoot elongation and proliferation in response to 10 mg/ℓ ZR

Fig. 27 - Shoot proliferation in a submerged culture

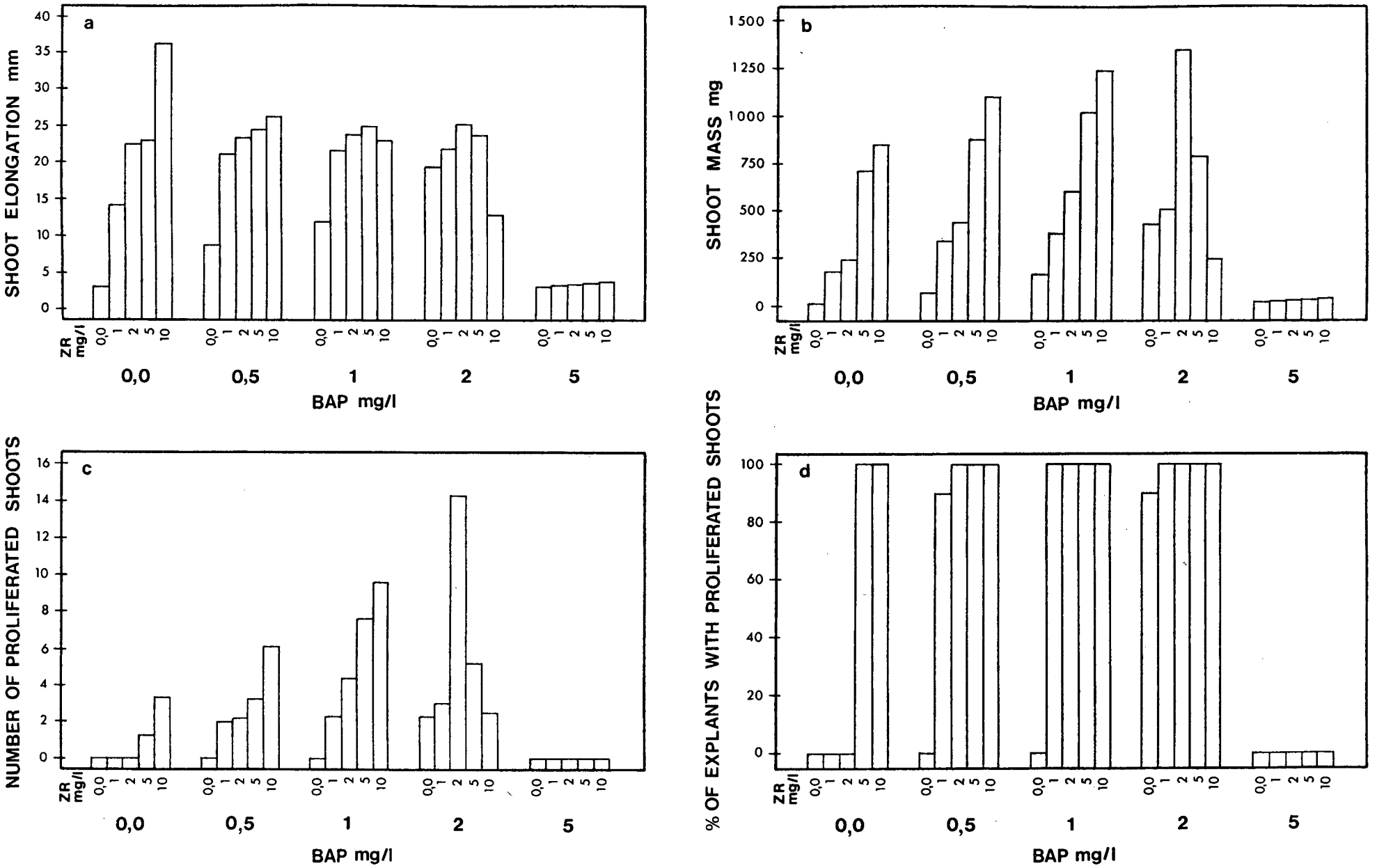


Fig. 28 - The effect of different concentration ratios of 6-benzylaminopurine (BAP) and zeatin riboside (ZR) on shoot elongation (a), total shoot mass (b), number of proliferated shoots (c), and percentages of explants with proliferated shoots (d), derived from shoot apices of Chenin cultured in vitro

4.7 Effects of cytokinins in routine subculturing

4.7.1 Objective and procedure

The most substantial commercial use of *in vitro* techniques has been in rapid clonal multiplication (Murashige, 1977; Hussey, 1980; Wang and Hu, 1980). In previous experiments (4.3, 4,4, 4.5, 4.6) responses to BAP and ZR of excised shoot apices of Chenin were investigated within single culture periods, excluding subsequent transfers to fresh media. Objectives of this study were the effects of the latter cytokinins in routine subculturing on the rate of proliferation and morphological characteristics of proliferated shoots.

The procedure, except where specified, was according to section 3, using the cv. Chenin. For initial culturing 10 mg/ℓ ZR was used. ZR was included at such high levels (initially and in subcultures) because of its previously observed marked stimulation of elongation and proliferation of shoots from excised apices. After 30 days, single cultures showing vigorous shoot elongation and proliferation (each having at least five shoots > 5 mm) were transferred from individual test tubes to 500 mℓ wide-mouth Erlenmeyer flasks containing 80 mℓ of medium. Subculture media were enriched with: (i) 2 mg/ℓ BAP; (ii) 2 mg/ℓ BAP + 2 mg/ℓ ZR and (iii) 10 mg/ℓ ZR. Control flasks lacked cytokinins. Each treatment consisted of five flasks replicated twice. Cultures were repeatedly transferred to fresh media at 15-day intervals. At each subculture shoot clumps were subdivided into more or less equal parts after proliferated shoots (> 5 mm) had been excised. The experiment was terminated after

five subcultures. Data were subjected to analysis of variance followed by Duncan's multiple range test (Snedecor and Cochran, 1967).

4.7.2 Results

The results are presented in Figs. 29 - 35.

Cytokinin application in subculture resulted in considerable shoot proliferation (Fig. 29). Maximum shoot production (significant at the 5% level) was induced by a combination of BAP and ZR (both at 2 mg/ℓ). Up to the second subculture, shoot proliferation was relatively limited. Subsequently, it increased almost linearly (Figs. 30, 31). Treatments lacking cytokinins (control) produced no shoots in subsequent subcultures.

Extensive submerged shoot proliferation was observed with BAP singularly and in combination with ZR at the concentrations applied (Fig. 32). Submerged shoot proliferation may account for higher overall shoot frequency as well as for sharp increases in the number of shoots over 15-day intervals. With 10 mg/ℓ ZR no submerged shoot proliferation occurred. In subsequent subcultures shoot clumps responded after 35 - 40 days to high levels of ZR (10 mg/ℓ) with submerged callus growth. A compact, nodular, greenish callus tissue resulted, which was completely absent with 2 mg/ℓ BAP, either alone or in combination with low concentrations of ZR (2 mg/ℓ).

Shoots produced with 2 mg/ℓ BAP, singularly and in combination with 2 mg/ℓ ZR, lacked uniformity of size, appeared shorter and

thicker and exhibited curved growth patterns. Leaves were conspiciously abnormal (Fig. 33). Dichotomous-like branching of proliferated shoots occurred; it was especially discernible after 4 - 5 days following transfer to fresh media (Fig. 34). Although after 9 - 10 days the apical regions of many shoots were forked, subsequent elongation was limited. Continuous branching of proliferated shoots gave rise to the formation of dense shoot clusters with numerous protruding shoots.

When applied in combination with BAP, low concentrations of ZR (2 mg/ℓ) did not alter the morphological characteristics of proliferated shoots. It is not clear whether this response could be specifically attributed to BAP or whether it was caused by gross shoot proliferation. Shoots produced with 10 mg/ℓ ZR were characterized by single axes, were slim, erect, more uniform in size, bore almost typical leaves, elongated rapidly, lacked any branching and resulted in shoot clumps of lower density (Fig. 35). Shoot production with the latter treatment appeared to be mainly due to development and growth of axillary meristems.

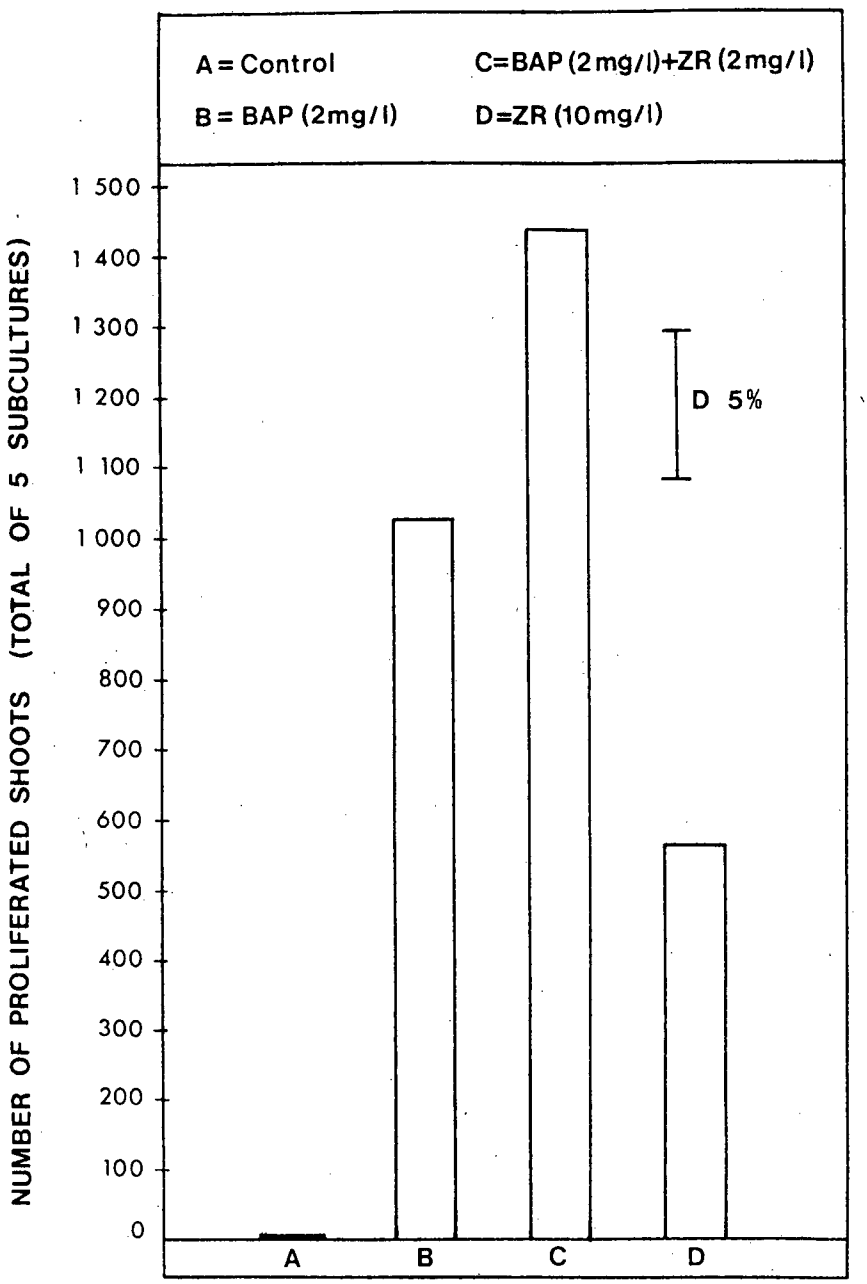


Fig. 29 - The effect of different concentration ratios of 6-benzylaminopurine (BAP) and zeatin riboside (ZR) in subculture on shoot proliferation from excised shoot apices of Chenin

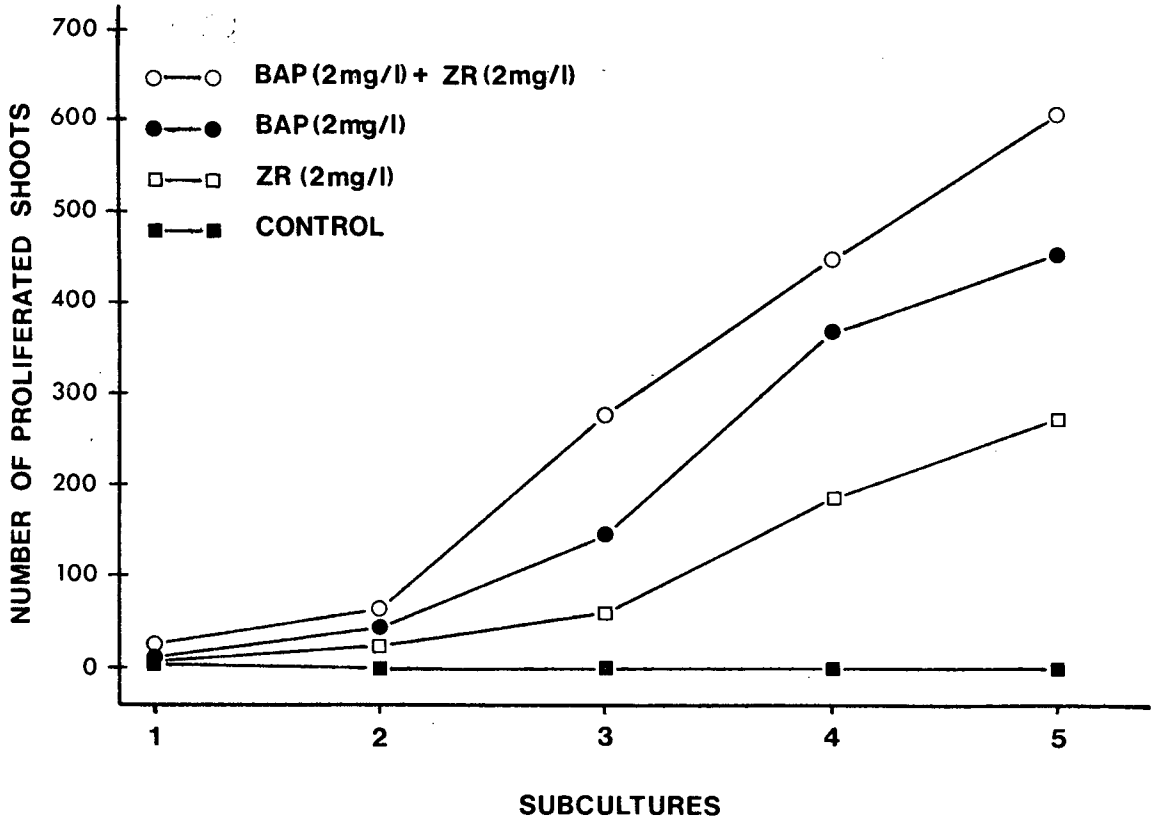


Fig. 30 - Shoot production over 5 subcultures in response to different concentrations of cytokinins

Fig. 31 - Shoot proliferation with 10 mg/ℓ ZR at 10 days after the second subculture.

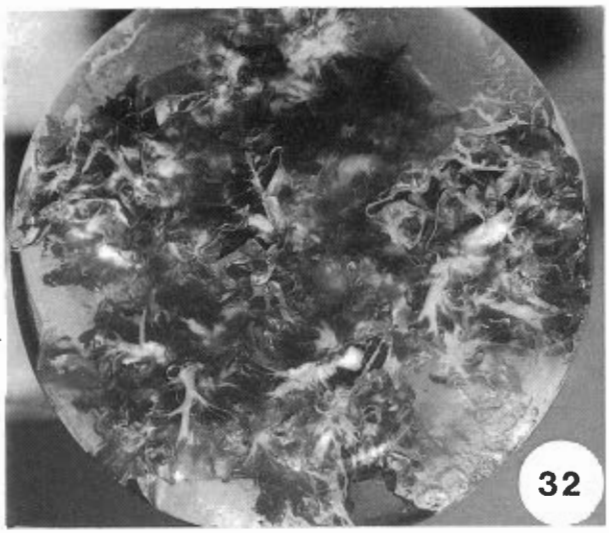
Fig. 32 - Submerged shoot proliferation with 2 mg/ℓ BAP at 12 days after the second subculture.

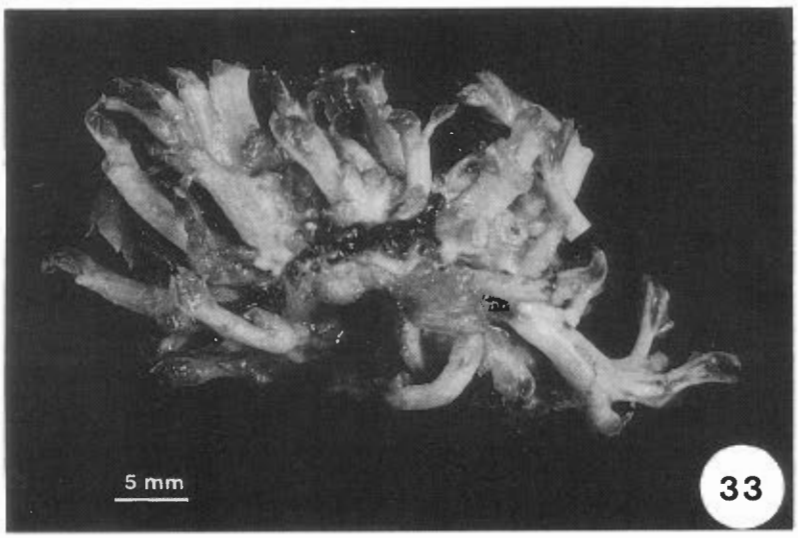
Fig. 33 - Morphological characteristics of shoots proliferated with 2 mg/ℓ BAP alone and in combination with 2 mg/ℓ ZR.

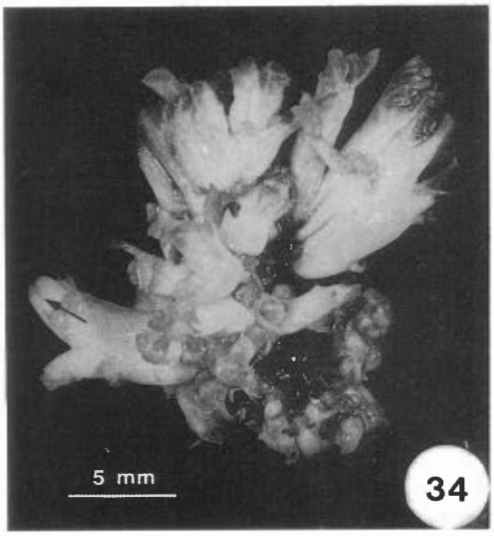
Fig. 34 - Enlarged part of shoot clump in Fig. 33 showing dichotomous-like branching (arrowed).

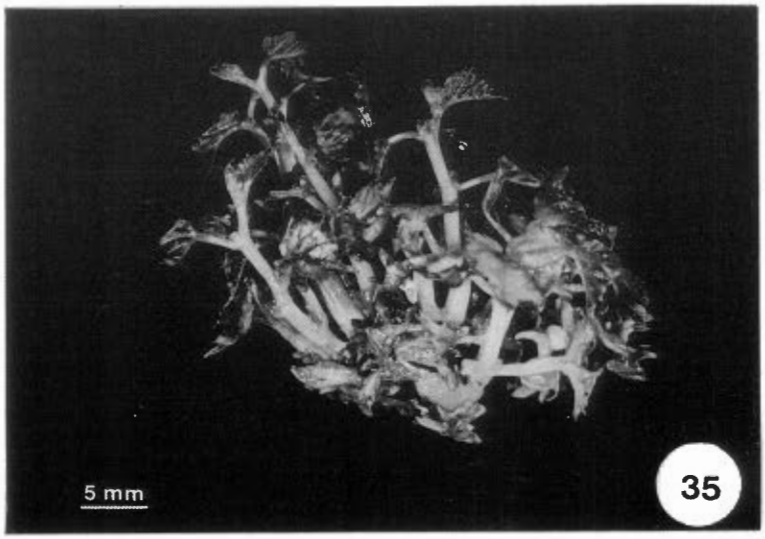
Fig. 35 - Morphological characteristics of shoots proliferated with 10 mg/ℓ ZR.

31









4.8 Discussion

4.8.1 Cytokinin effects

Irrespective of the presence of cytokinins in actively growing shoot tips of grapevines (Volmer, 1976), results of the present investigation clearly indicate that apices, once excised, were dependent on external sources of cytokinin in order to respond with *in vitro* leaf expansion, shoot elongation and proliferation. This is in accordance with reports for other woody species (Jacobs *et al.*, 1969; Elliot, 1970; Jacobs, 1973; Abbott and Whiteley, 1976; Jones *et al.*, 1977; Tabachnik and Kester, 1977; Broome and Zimmerman, 1978; Chaturvedi *et al.*, 1978; Hammerschlag, 1980; Singha, 1980).

Although the cytokinins tested were active over a range of concentrations, optimum levels varied greatly between different cytokinins. Low concentrations of BAP (0,1 - 2 mg/ℓ) stimulated shoot elongation and proliferation but were inhibitory at higher levels (3,5 - 5 mg/ℓ). This supports the findings of Jones (1977) for apples, Adams (1975) for hops, Mekers (1977) for ornamental bromeliad cultivars, Hammerschlag (1980) for peaches and Singha (1980) for pears. Although Preil and Engelhardt (1977) reported that shoot proliferation of Azaleas was stimulated with increasing concentrations of BAP (2 - 5 mg/ℓ), lower BAP levels (0,1 - 2 mg/ℓ) appear to be optimal for most woody species. Both zeatin and ZR elicited enhanced shoot elongation and proliferation, with concomitant increases in mass. Optimal rates were, however, achieved at much higher levels if compared with BAP.

Over the concentration ranges tested, kinetin appeared to be the least effective for shoot elongation and proliferation of cultured shoot apices, in comparison with BAP, zeatin and ZR. It is, however, possible that the concentrations of kinetin tested were further from the optimum than for the other three cytokinins.

4.8.2 Cytokinin:Auxin effects

The effects of kinetin on shoot elongation, increases in mass and leaf expansion were enhanced when used in combination with IAA, provided IAA concentrations did not exceed 8 mg/ℓ for Chenin and 4 mg/ℓ for 1-9. In the presence of relatively high IAA concentrations, shoot elongation was either slightly inhibited or retarded in contrast to slight stimulatory effects of lower levels. Applications of both NAA and IBA singly strongly inhibited shoot elongation and leaf expansion. In combination with kinetin, inhibitory effects were only slightly overcome at appropriate levels.

Excised shoot apices of some herbaceous plants responded with marked shoot proliferation to kinetin:auxin combinations (Ben-Jaacov and Langhans, 1972; Jelaska and Sutina, 1977; James and Newton, 1977). In the present investigation the addition of kinetin, whether alone or in combination with IAA, NAA or IBA, failed to promote axillary shoot production.

Naphthaleneacetic acid in some cases suppressed stimulatory effects of both BAP and ZR on shoot elongation. In Chenin, higher yields in shoot mass with BAP (0,5 and 1 mg/ℓ) and ZR (5 and

10 mg/ℓ) in combination with 0,1 mg/ℓ NAA (although not always statistically significant) may be attributed to diameter increase of stem parts and increased rates of shoot proliferation. Shoot proliferation rates were influenced by an interaction between 5 or 10 mg/ℓ ZR and 0,1 mg/ℓ NAA (Chenin). However, the occurrence of callus growth with 5 and 10 mg/ℓ ZR in combination with 0,1 mg/ℓ NAA may be considered as a disadvantage, due to the probability of shoot clusters changing ultimately into callus-like masses (Murashige, 1977; Hussey, 1980). Apart from 0,1 mg/ℓ NAA in combination with 2 mg/ℓ BAP (Chenin) and with 10 mg/ℓ ZR (both cultivars), shoot proliferation in all cultures (100%) did not occur with other combinations.

No callusing occurred on media lacking auxin. Meristematic cells of explants apparently do not contain sufficient auxin to promote callus growth when cultured *in vitro*. In the absence of cytokinin, NAA promoted callus initiation and growth, whereas IAA and IBA did not. In culturing young green stem segments of grapevine *in vitro* (Orffer, 1956), callus initiation was observed to be favoured by high humidity, but if water and agar media were in contact with exposed wounds, callus initiation was suppressed. Suppressive effects of liquid media on callus growth irrespective of the presence of auxin was overcome by the presence of adenine sulphate (Orffer, 1956). Suppressed callus growth in the present investigation irrespective of the presence of IBA and IAA may thus be correlated with the orientation of explants on the media surface as on all occasions exposed wounds were in contact with agar. Nevertheless, the re-

sults clearly indicate that callusing was influenced by interactions between cytokinins and auxins, especially discernible in combinations of kinetin with NAA and IBA and BAP and ZR in combination with NAA. Combined applications of kinetin and IAA, however, failed to induce callus initiation. The inability of IAA alone, as well as in combination with kinetin to initiate callus, may also be due to the low activity and stability of IAA when applied in culture (De Fossard, 1976; Hussey, 1980). Although not statistically analysed, 1-9 responded to a much higher degree with callus growth than Chenin to the applied cytokinin:auxin ratios.

Suppressive effects of auxin on root formation of detached shoot apices are in contrast to the findings for other plant species (Stone, 1963; Van Os, 1964; Jones and Vine, 1968; Hollings and Stone, 1970; Jacobs, 1973; Boxus, 1975). Although in grapevines rooting of small excisions of apices occurred in the presence of auxin (Gifford and Hewitt, 1961; Galzy, 1972; Hoeffer and Gifford, 1964; Bini, 1976), recovery percentages were very low. Barlass and Skene (1978, 1979) reported successful rooting of *in vitro* produced shoot tips (> 3 mm) on media lacking auxin. Elongating shoot tips apparently do contain sufficient auxin to promote rooting when cultured *in vitro*.

Since rooting in the present investigation was not stimulated in the presence of cytokinin alone, results with ZR in combination with NAA indicated that an interaction between these growth substances exists with respect to root formation in Chenin. Rooting occurred in all combinations of 0,1 - 2 mg/ℓ NAA with

1 - 5 mg/ℓ ZR. In contrast to the general agreement that root formation is favoured by high auxin/cytokinin relationships (Murashige, 1977), the highest levels of NAA combined with the lowest levels of ZR lacked root initiation. Although maximum rooting resulted with 1 mg/ℓ NAA in combination with 2 mg/ℓ ZR, root elongation was suppressed with this treatment. Inhibitory effects of auxin on root elongation are in accordance with reports for other plant species (Murashige, 1977; Cheng, 1978; Hussey, 1980; Allan, 1981a,b). Root elongation achieved with 0,1 mg/ℓ NAA in combination with 1 - 5 mg/ℓ ZR may perhaps be attributed to stimulatory effects of the cytokinin. However, elongated roots with the above combination did not contribute to a stimulation of shoot elongation within the culture period of 42 days.

The above findings emphasize that the proper choice of cytokinin and auxin and the adjustment of concentrations of the two growth substances is of major importance for detached grapevine shoot apices to respond maximally when cultured *in vitro*.

4.8.3 Cytokinin:Gibberellin effects

Stimulatory effects of GA_3 alone on primary shoot elongation of excised shoot apices resemble the findings of other workers (Morel, 1964a,b; Jones and Vine, 1968; Vine and Jones, 1969; Earle and Langhans, 1974; Jacob, 1974). Shoot elongation was, however, stimulated only to a limited extent and tiny shoots bearing thin, malformed leaves resulted.

Jacobs (1970a) reported that the favourable effect of kinetin on leaf expansion in rose cv. Superstar was reduced by GA_3. In the present investigation the activity of ZR on shoot elongation and leaf expansion was enhanced when used in combination with GA_3, provided GA_3 concentrations did not exceed 1 mg/ℓ for 1-9.

Singha (1980) and Zimmerman and Broome (1980) used cytokinins in combination with GA_3 for multiple shoot production from detached shoot apices of pears and blackberries respectively. No inhibitory effects on axillary shoot growth or survival rates were reported. In the present investigation it was observed - not shown in the results - that ZR alone, as well as in combination with GA_3, did not promote axillary shoot production (even on shoots bearing several leaves). The absence of axillary shoots with ZR treatments may be attributed to the shortened culture period of 18 days. Tendril formation in the presence of GA_3 was not influenced with combined applications of ZR.

4.8.4 Cytokinin:Cytokinin effects

The activity of BAP as well as ZR on shoot elongation and proliferation of excised shoot apices cultured *in vitro* was enhanced when used in combination at certain ratios. Stimulation of shoot elongation in combinations of 0,5 - 2 mg/ℓ BAP with 1 - 5 mg/ℓ ZR is probably due to the favourable effect of ZR at relative high concentrations. Although shoot elongation was reduced with combinations of 1 mg/ℓ BAP and 10 mg/ℓ ZR in relation to 1 mg/ℓ BAP and 5 mg/ℓ ZR, mass increased; however, not statistically significant. This may be attributed to an

increase in leaf expansion, axillary shoot production and diameter increase of stem parts.

The results of the present study clearly indicated that, although shoot proliferation was achieved with single applications of BAP (2 mg/ℓ) and ZR (5 and 10 mg/ℓ), interactions between the cytokinins occur, especially discernible with a combination of 2 mg/ℓ BAP and 2 mg/ℓ ZR. Inhibitory effects of BAP at 5 mg/ℓ on both shoot elongation and proliferation could, however, not be alleviated with combined ZR treatments (1 - 10 mg/ℓ).

4.8.5 Routine subculturing

Cytokinin application in routine subculturing proved highly effective for multiple *in vitro* shoot production from a single excised apex. Although ZR at 10 mg/ℓ resulted in lower quantities of proliferated shoots if compared with BAP (alone at 2 mg/ℓ or in combination with 2 mg/ℓ ZR), the morphological characteristics resembled those of the normal condition. It was indicated, however (Barlass and Skene, 1978, 1979), that once rooted, shoots produced with BAP in the medium developed into normal plants following passage through a "juvenile" phase (i.e. spiral phyllotaxy and absence of tendrils). With rapid propagation of grapevine clones as a goal, cytokinin application in routine subculturing of shoop clumps derived from shoot apices would be an effective tool.

SECTION 5

ANATOMICAL RESPONSES TO CYTOKININS OF ABSCISED SHOOT APICES OF GRAPEVINES CULTURED IN VITRO

5.1 Introduction

The anatomical structure of grapevine shoot apices and differentiation pathways of vascular tissues in elongating shoots have been described by Branas, 1957; Hegedüs, 1957; Thompson and Olmo, 1963; Tucker and Hoefert, 1968, Fournioux, 1972 and Pratt, 1974.

With rapid propagation of grapevine *in vitro* it is of extreme importance that proliferated shoots derived from detached apices should lead to the production of genetically uniform plants. In general, multicellular organized shoot meristems are genetically highly stable (Murashige, 1974; Abbott, 1978; Hussey, 1980). Although adventitious meristems may enable a substantially faster cloning rate than axillary shoot multiplication, wholly mutant propagules may arise, due to growth from single or very small groups of cells (Hackett and Anderson, 1967; Malnassy and Ellison, 1970; Ben-Jaacov and Langhans, 1972; Murashige, 1977; Anderson, 1980; Hussey, 1980; Wang and Hu, 1980; Allan, 1981a,b).

Barlass and Skene (1978, 1980) and Barlass *et al.* (1981) demonstrated with light and scanning electron microscopy that shoots derived *in vitro* from fragmented grapevine shoot apices are adventitious in origin. In section 4 it was shown that shoot

proliferation derived from non-fragmented apices of Chenin was enhanced by the addition of cytokinins to nutrient media.

This study was undertaken to investigate: (i) anatomical responses of excised grapevine shoot apices to cytokinin application *in vitro* and (ii) whether *in vitro* produced shoots are of axillary or adventitious origin.

5.2 Procedure

Excised shoot apices of Chenin were cultured as described in section 3. Anatomical responses of explants to BAP (2 mg/ℓ); BAP + ZR (both at 2 mg/ℓ) and ZR (10 mg/ℓ) were studied at various intervals (days) after the start of culture. Control treatments lacked cytokinins. Sections were also prepared of shoot tips (5 mm) taken from elongating shoots on cane segments of Chenin.

For light microscopy (LM), tissues were fixed in formalin-acetic acid-alcohol (FAA), infiltrated by means of a tertiary butanol-paraffin wax procedure (Johansen, 1940) and sectioned with a rotating microtome. Sections (8,0 µm) were stained utilising the tannic acid, safranine, fast-green technique (De Vos, 1974).

Preparation of material for scanning electron microscopy (SEM) was according to Hayat (1974). *In vitro* produced shoots were fixed in 25% glutaraldehyde (pH 4) for 24 h at 4°C, washed thrice for 30 min periods in a 0,2 M sodium cacodylate buffer (pH 7,2) and dehydrated in a graded acetone-water series. The

material was then subjected to crytical-point drying with CO_2 and sputter-coated with gold, after which it was studied with an ISI - 100A scanning electron microscope.

5.3 Results

The anatomical structure of the apical nodes of an elongating shoot of Chenin with initiation of leaf and tendril primordia and origin of axillary meristems is presented in Fig. 36. The apical meristem of the shoot (AMS) consisting of two tunica layers over a poorly defined corpus is shown in Fig. 37. A leaf primordium (L1) is initiated on the flank of the AMS by periclinal divisions in the second layer.

Culture media lacking cytokinins resulted in limited elongation of exised shoot apices. Serial sections obtained at 40 days after the start of culture showed leaf and tendril initiation on the flanks of the AMS (Fig. 38). However, subsequent development of the youngest leaves and tendrils was reduced. Primary vascularization was observed acropetally to No. 5 leaf bases (Fig. 39). Over the culture period of 45 days explants lacked initiation of axillary meristems.

After 10 days' culture, shoot apices responded with marked elongation and concomitant leaf and tendril initiation to the range of cytokinins applied (Fig. 40). The typical discontinuous arrangement of tendrils of Vitis (exception V. labrusca) persisted. Sieve elements and spirally thickened vessels differentiated

at the base of leaf 3 (Fig. 41). The anatomical structure of the AMS of shoots resembled that of elongating shoots on established vines (Fig. 42). Procambial traces are well-marked (leaf 2). The youngest axillary meristem originated in the axil of leaf 3.

In response to 2 mg/ℓ BAP, singly and in combination with 2 mg/ℓ ZR, the apical part of the primary shoot increased in diameter, with the apical meristem less prominently elevated above the youngest tendril and axillary meristem at 20 days after the start of culture (Figs. 43, 44). After 25 days of culture, apical dominance was overcome, expressed by the strong development of the axillary meristem nearest to the AMS (Fig. 45). Pronounced vascularization occurred. Sieve elements and spirally thickened vessels differentiated at the base of leaf 2 (Fig. 46).

Shoot clumps with several protruding shoots resulted at 35 days after the start of culture on media enriched with 2 mg/ℓ BAP (singly and in combination with 2 mg/ℓ ZR) (Figs. 47, 48). Light and scanning electron microscopy of clumps revealed that some shoot apical meristems appeared practically at one level with axillary meristems (Figs. 49, 50), or in a slight depression below it (Fig. 51). This phenomenon was especially discernible in response to applications of BAP at 2 mg/ℓ. Once released from apical dominance, the apical meristem of the axillary shoot duplicated the pattern found in the main AMS and proceeded with initiation of leaves and axillary meristems (Fig. 52). Axillary

shoots, however, lacked tendril initiation. Subsequent elongation of axillary shoots in response to BAP (2 mg/ℓ) alone was limited (Fig. 53). With a combination of BAP and ZR (both at 2 mg/ℓ) elongation of axillary shoots was slightly more pronounced, with origination and subsequent development of axillary meristems at lower levels down the axes (Fig. 54). Leaf expansion on axillary shoots with the occurrence of abundant stomata is shown in Fig. 55.

Although apical branching of proliferated shoots in response to 2 mg/ℓ BAP (singly and in combination with 2 mg/ℓ ZR) was observed at earlier stages during culture, it occurred at increased levels after 40 days (Fig. 56). The apical regions of many shoots became forked (Figs. 57, 58) with organized apical meristems. They elongated with subsequent initiation and development of axillary meristems in leaf axils. However, in response to BAP (2 mg/ℓ) alone, elongation of forked shoots was limited and coupled with a strong release of the youngest axillary meristems from apical dominance (Fig. 59). In combination with ZR (2 mg/ℓ) elongation was more pronounced with axillary meristems originating at slightly lower levels down the axes (Fig. 60). However, once released from apical dominance, development and growth of these axillary meristems followed the main AMS closely (Figs. 61, 62). Continuous axillary branching of proliferated shoots coupled with a strong release of axillary meristems from apical dominance gave rise to the formation of dense shoot clusters with numerous proliferating shoots (Fig. 63).

Excised shoots apices responded markedly to the application of ZR at high concentrations (10 mg/ℓ). After 10 days' culture the anatomical structure of explants resembled that observed with 2 mg/ℓ BAP (singly and in combination with 2 mg/ℓ ZR). Marked elongation at 20 days after the start of culture with concomitant leaf initiation and origination of axillary meristems is shown in Fig. 64. Elongating shoots lacked tendril initiation. After 25 days' culture the AMS still appeared well elevated above the youngest defineable leaf and axillary meristem (Fig. 65). Axillary meristems nearest the AMS lacked active development and elongation. Primary vascularization proceeded strongly (base of leaf 3). Marked differentiation of sieve elements and spirally thickened vessels occurred (Fig. 66). After 30 days of culture, axillary meristems at lower levels down the axis of the primary shoot responded with pronounced development and elongation (Fig. 67). Proliferated shoots lacked any branching and resulted in shoot clumps of low density, even at 40 days after the start of culture. However, they elongated rapidly and elicited long internodes. Stretching of xylem elements occurred as expressed by the uncoiling of spirals (Fig. 68).

5.4 Discussion

The structure of the apical part of an elongating shoot of Chenin with leaf and tendril initiation, axillary meristem origination and primary vascularization agrees with descriptions of Hegedüs, 1957; Thompson and Olmo, 1963; Tucker and Hoefert, 1968; Fournioux, 1972 and Pratt, 1974.

Results of the present investigation stress the dependency of detached grapevine shoot apices on the presence of an external source of cytokinin to elongate, with a concomitant production of leaves, each of which carries in its axil a replica of the main apex. Upon release from apical dominance, these axillary meristems were capable of producing their own axillary meristems. The potential of cytokinins to stimulate development and elongation of axillary meristems, thus inducing in vitro shoot multiplication, is in agreement with reports for other woody species (Abbott and Whiteley, 1976; Jones et al., 1977; Quoirin and Lepoivre, 1977; Tabachnik and Kester, 1977; Broome and Zimmerman, 1978; Chaturvedi et al., 1978; Cheng, 1978).

Shoots derived from fragmented grapevine shoot apices were adventitious in origin (Barlass and Skene, 1980; Barlass et al., 1981). In the present investigation no evidence was found that proliferated shoots originated adventitiously. Dense shoot clusters were induced by the enhanced release of axillary meristems from apical dominance, due to the application of BAP at 2 mg/ℓ alone, as well as in combination with 2 mg/ℓ ZR. Axillary meristems on these axillary shoots were subsequently released from apical dominance, thus giving rise to dense clusters with the main AMS occasionally encircled by developing axillary meristems.

Branching of the apical parts of shoots protruding from these clusters could be traced to the strong release from apical dominance of axillary meristems nearest to the main AMS. Due

to strong development and elongation of these axillary meristems, fork-like branches resulted. Subsequent branching contributed to the formation of clusters of variable densities. Shoots produced with 10 mg/ℓ ZR lacked any branching and elongated rapidly. However, elongation of axillary meristems nearest to the main AMS was initially limited. Shoot clusters of low densities may be attributed to pronounced elongation of axillary meristems at lower levels down the axes.

Explants cultured on cytokinin-enriched media proceeded with vascularization according to the pathways as described for growing shoot tips of established vines (Hegedüs, 1957; Fournioux, 1972; Pratt, 1974). In the present investigation a tendency for sieve elements and vessels to differentiate at levels nearer to the main AMS was observed, especially in shoot clumps of high densities.

It is concluded that proliferated shoots derived from non-fragmented grapevine shoot apices is of axillary origin. Applications of BAP at 2 mg/ℓ, alone or in combination with 2 mg/ℓ ZR, resulted into more pronounced overcoming of apical dominance than 10 mg/ℓ ZR.

Fig. 36 - Apical nodes of elongating shoot from a cane segment of Chenin showing apical meristem (AM), leaf (L) and tendril (T) primordia and axillary meristems (Ax M). (Numbers indicate basipetal sequences). (LM x 64).

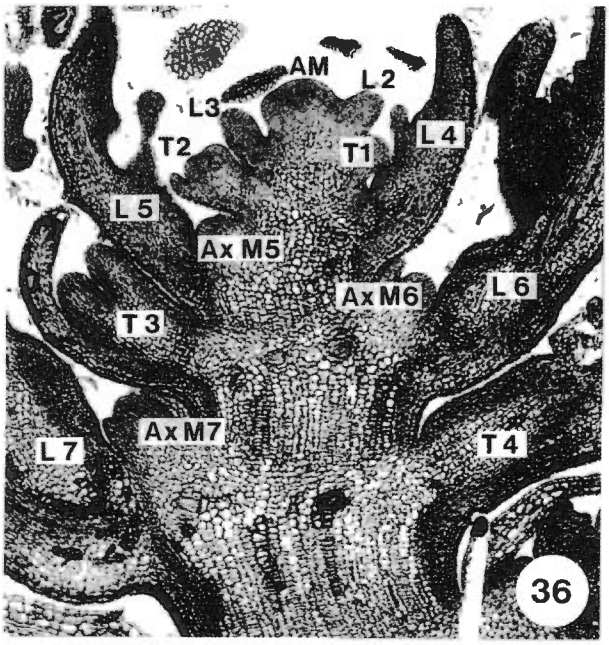
Fig. 37 - Shoot apex in Fig. 36 enlarged to show apical meristem (AM), 2-layered tunica (Tu), corpus (Corp), rib meristem (RM), diaphram (D), leaf initiation (L1) and leaf (L) and tendril (T) primordia. (LM x 350).

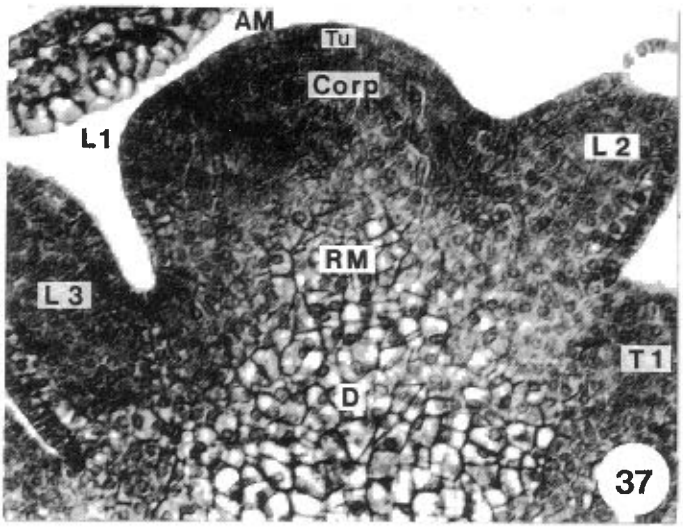
Fig. 38 - Shoot apex of Chenin after 40 days of culture on media lacking cytokinins showing apical meristem (AM), 2-layered tunica (Tu), corpus (Corp), leaf initiation (L1) and leaf (L) and tendril (T) primordia. (LM x 210).

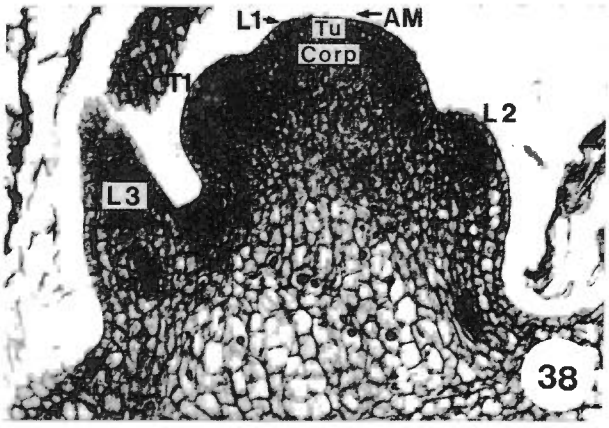
Fig. 39 - Apical part of shoot in Fig. 38. Arrow indicates primary vascularization acropetally to No. 5 leaf base. (LM x 130).

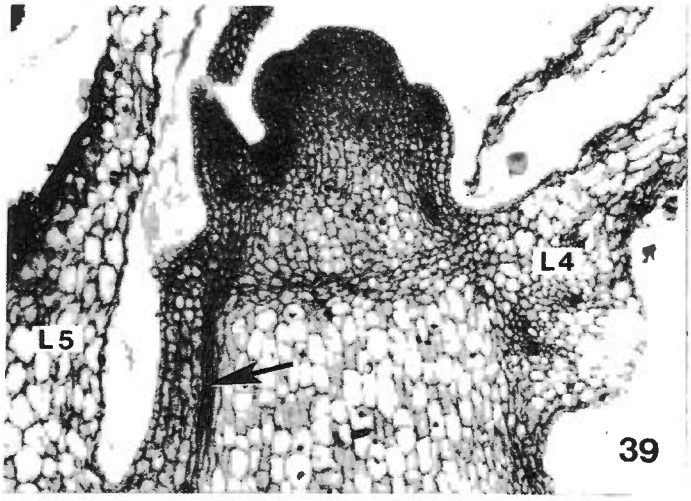
Fig. 40 - Elongation, subopposite initiation of leaf (L) and tendril (T) primordia and origination of axillary meristems (Ax M) on shoot derived from apex of Chenin after 10 days' culture on cytokinin-enriched media. Arrow indicates primary vascular differentiation. (LM x 87).

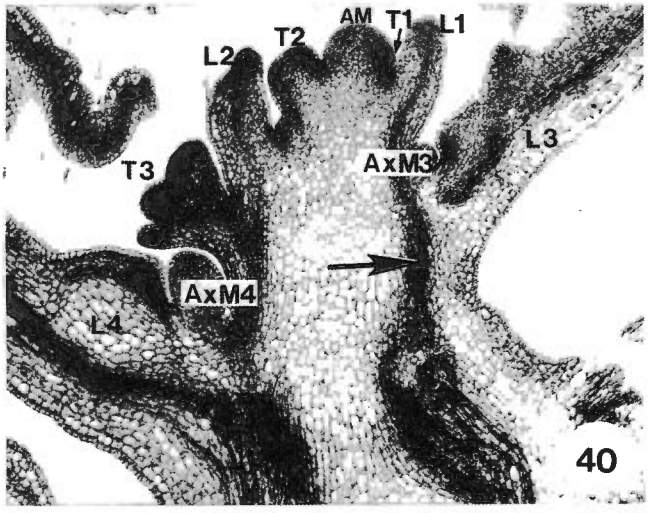
Fig. 41 - Differentiation of sieve elements (S) and spirally thickened vessels (V) at the base of leaf 3 (Fig. 40) after 10 days' culture of shoot apices of Chenin on cytokinin-enriched media. (LM x 490).











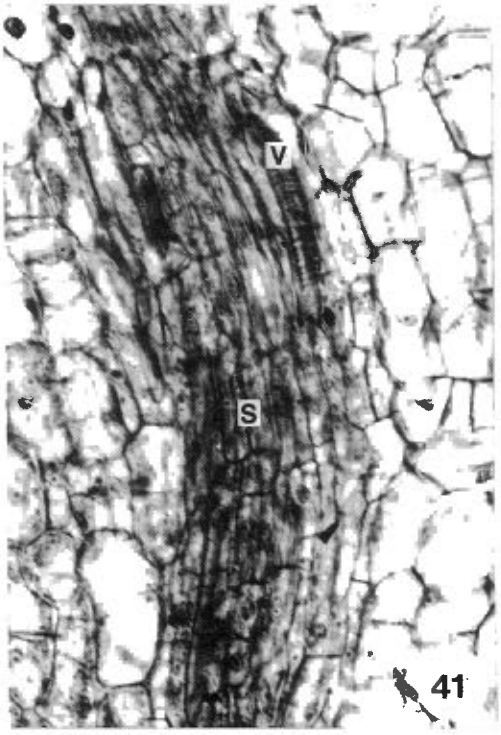


Fig. 42 - Shoot apex as in Fig. 40 at 10 days after the start of culture on cytokinin-enriched media showing the apical meristem (AM), 2-layered tunica (Tu), corpus (Corp), leaf buttress (Lb), leaf (L) and tendril (T) primordia and origination of axillary meristem (Ax M) in the axil of leaf 3. Arrow indicates procambial traces (leaf 2). (LM x 210).

Fig. 43 - Apical part of shoot derived from apex of Chenin at 20 days after the start of culture on media containing 2 mg/ℓ BAP. Light micrograph (LM) showing increase in diameter of stem part and apical meristem (AM) less prominently elevated above the youngest tendril (T) and axillary meristem (Ax M). (LM x 87).

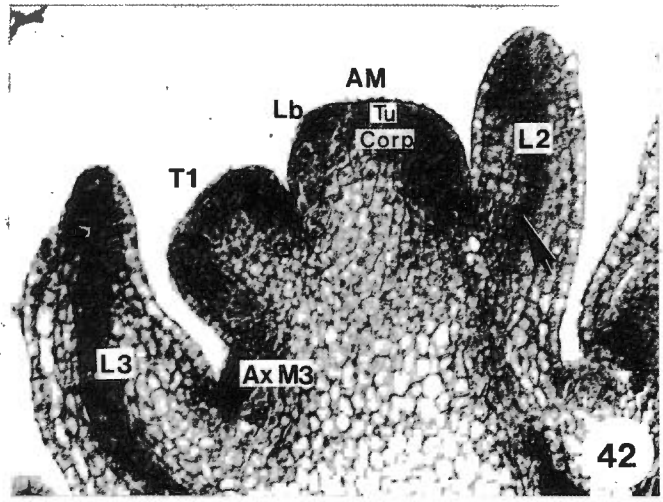
Fig. 44 - Shoot apex in Fig. 43 showing tendril (T) primordium and leaf (L) initiation on the flank of the apical meristem (AM). Arrow indicates leaf initiation on the flank of the axillary meristem (Ax M). (LM x 210).

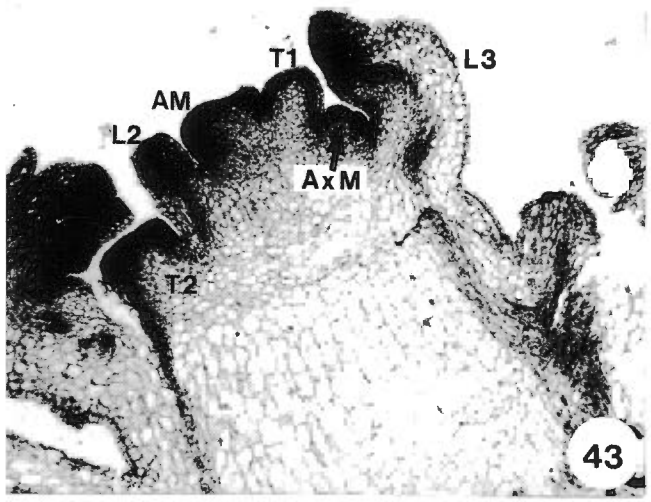
Fig. 45 - Apical part of shoot derived from apex of Chenin after 25 days' culture with 2 mg/ℓ BAP. Light micrograph (LM) showing leaf (L) and tendril (T) primordia and the overcoming of apical dominance as expressed by the strong development of the axillary meristem (Ax M) nearest to the apical meristem (AM). Arrows indicate leaf primordia on the axillary meristem (Ax M). Vascularization (Vs) proceeded strongly (base leaf 2). (LM x 105).

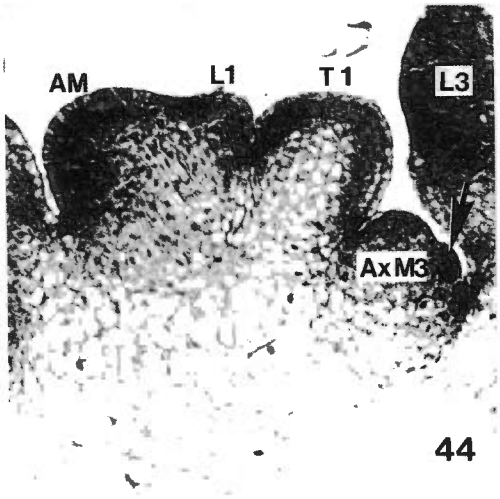
Fig. 46 - Differentiated sieve elements (S) and spirally thickened vessels (V) at the base of leaf 2 in Fig. 45. (LM x 250).

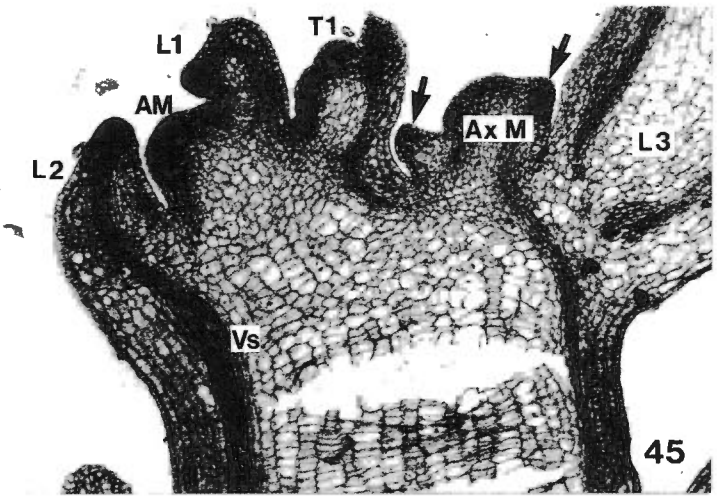
Fig. 47 - Part of shoot clump derived from shoot apex of Chenin at 35 days after the start of culture on media containing 2 mg/ℓ BAP, showing protruding shoots (PS). (LM x 87).

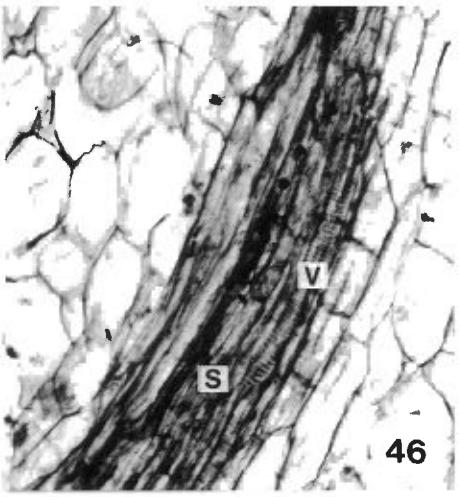
Fig. 48 - Part of shoot clump as in Fig. 47 showing protruding shoots (arrowed). (SEM x 25).

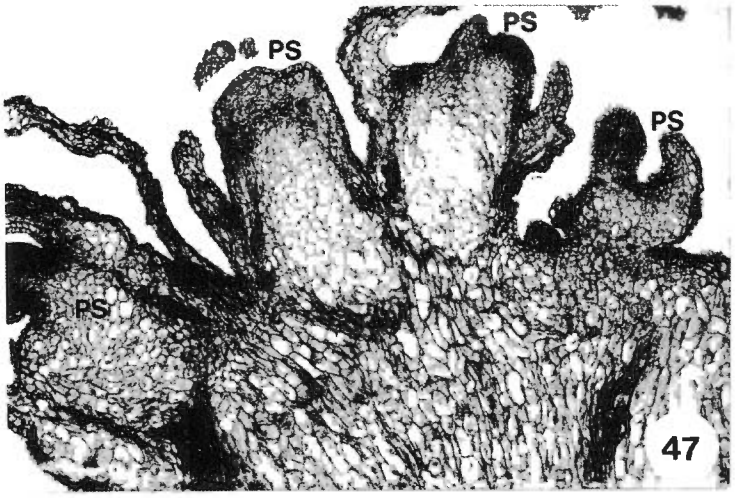












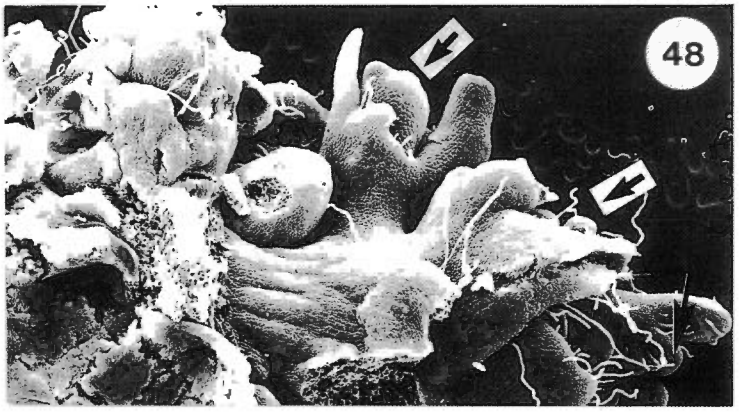


Fig. 49 - Proliferated shoot in clump as in Figs. 47 and 48 showing apical meristems (AM) to appear practically at one level with axillary meristems (Ax M). (LM x 135).

Fig. 50 - Proliferated shoot in clump as in Fig. 47 showing axillary meristem (Ax M) at the same level with the apical meristem (AM). (SEM x 180).

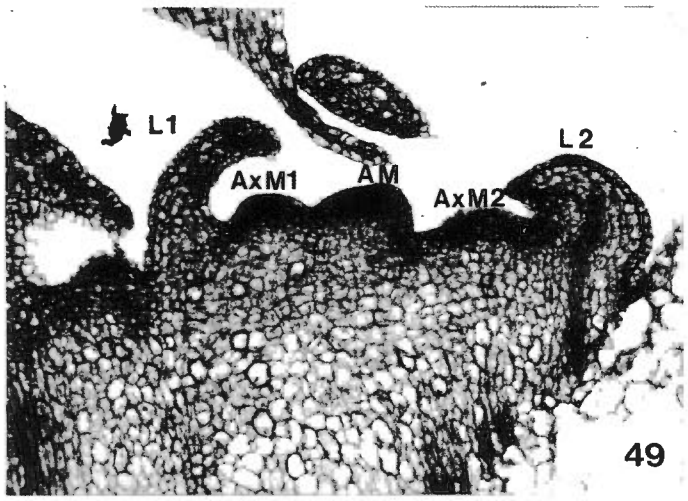
Fig. 51 - Proliferated shoot in clump showing shoot apical meristem (AM) in a slight depression below developing axillary meristem (Ax M). (LM x 140).

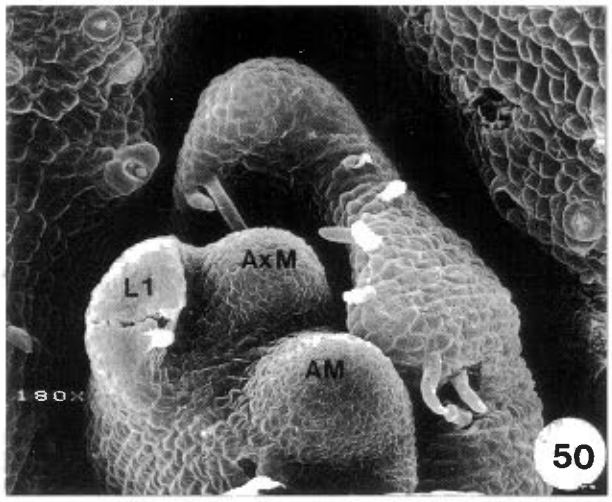
Fig. 52 - Apical part of axillary shoot once released from apical dominance with 2 mg/ℓ BAP, showing leaf (L) initiation, origination of axillary meristem (Ax M) as well as the occurrence of stomata (St) and the development of trichomes (Tc) from epidermal cells on leaf 2. (SEM x 180).

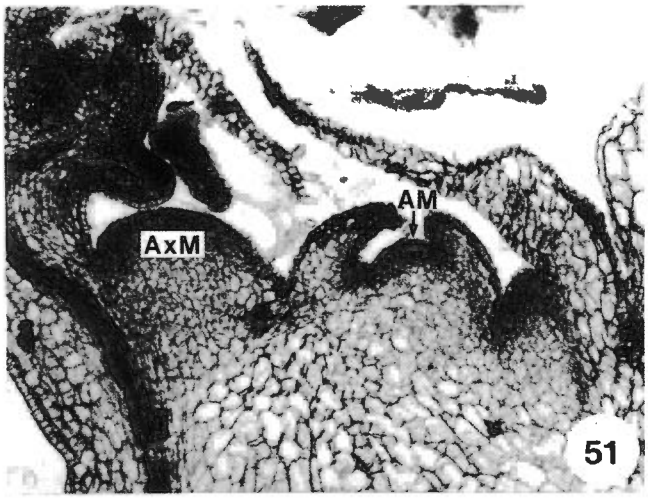
Fig. 53 - Axillary shoot once released from apical dominance with BAP (2 mg/ℓ) showing leaf (L) primordia and origination of axillary meristems (Ax M). (LM x 95).

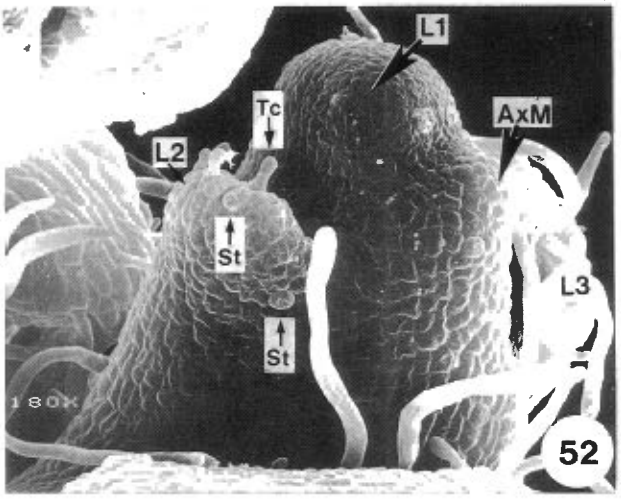
Fig. 54 - Axillary shoot once released from apical dominance with 2 mg/ℓ ZR plus 2 mg/ℓ BAP in the medium, showing the apical meristem (AM) and leaf (L) primordia. Asterix indicates leaf initiation on the flank of the apical meristem (AM). Elongation is more pronounced with origination and subsequent development of axillary meristems (Ax M) at lower levels down the axis. (LM x 80).

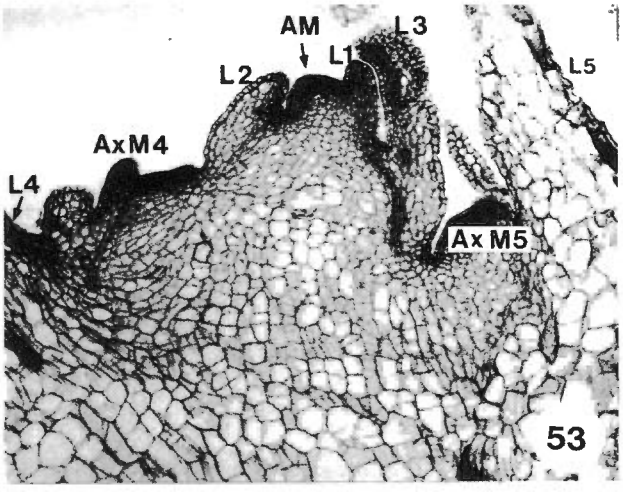
Fig. 55 - Leaf expansion on proliferated shoots. Arrows indicate the occurrence of stomata. (SEM x 60).











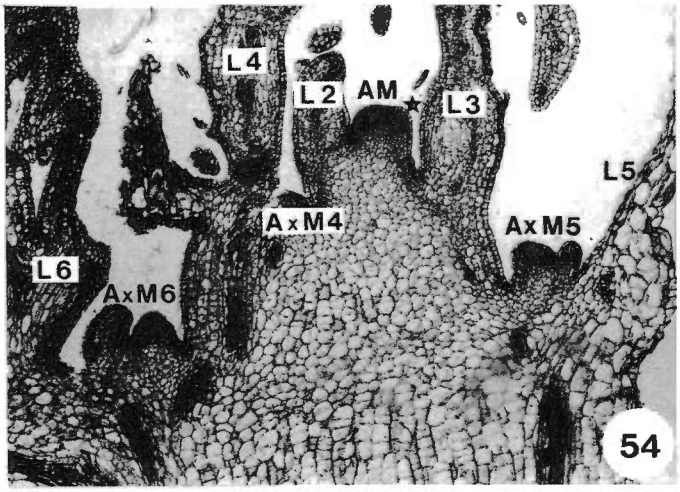


Fig. 56 - Branching of proliferated shoots (arrowed) in response to 2 mg/ℓ BAP, alone or plus 2 mg/ℓ ZR at 40 days after the start of culture. (SEM x 20).

Fig. 57 - Forking of apical regions of shoots with gradually organized apical meristems (AM) after 40 days of culture on media containing 2 mg/ℓ BAP alone. (LM x 82).

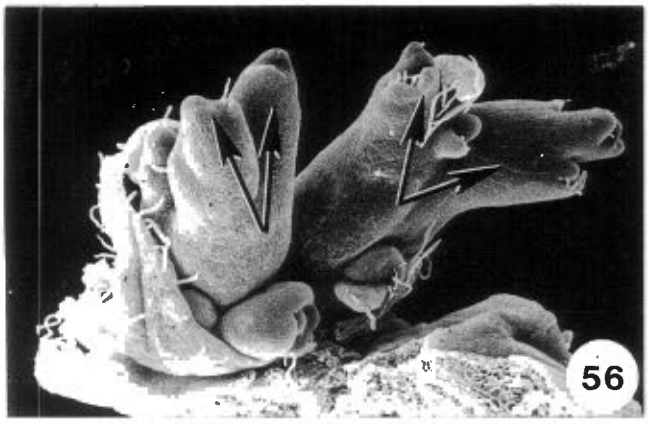
Fig. 58 - Forking of apical regions of shoots as in Figs. 56 and 57. The apical meristem (AM) of the shoot on the left is concealed by leaf (L) primordia. Axillary meristem (Ax M) with leaf primordium occurred in the axil of leaf 2 (shoot on the left). The apical meristem (AM) of the shoot on the right is well organized and proceeded with initiation of leaf (L) primordia. (SEM x 70).

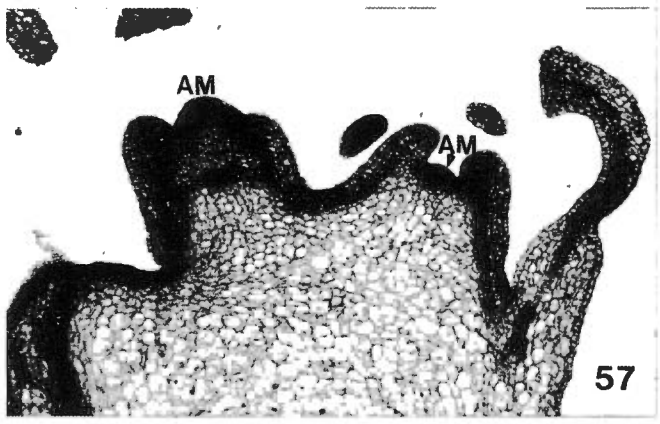
Fig. 59 - Limited elongation of shoots after forking at 40 days after the start of culture in response to BAP (2 mg/ℓ). Well organized apical meristems (AM) are coupled with a strong release of axillary meristems (arrowed) from apical dominance. (LM x 87).

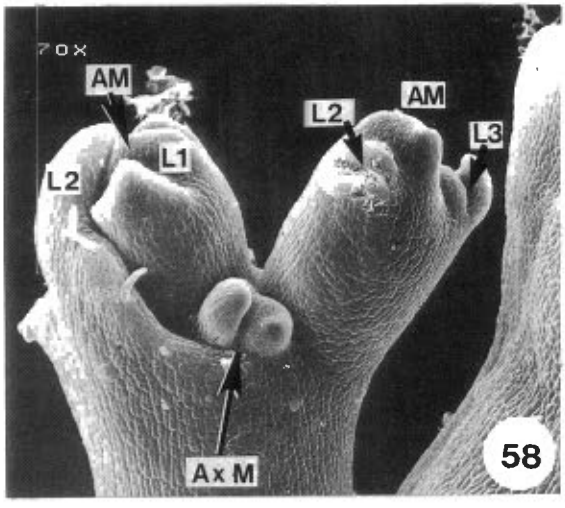
Fig. 60 - More pronounced elongation of shoots after forking with combinations of BAP and ZR (both at 2 mg/ℓ) at 40 days after the start of culture. Axillary meristems (arrowed) originated at lower levels down the axes. (LM x 95).

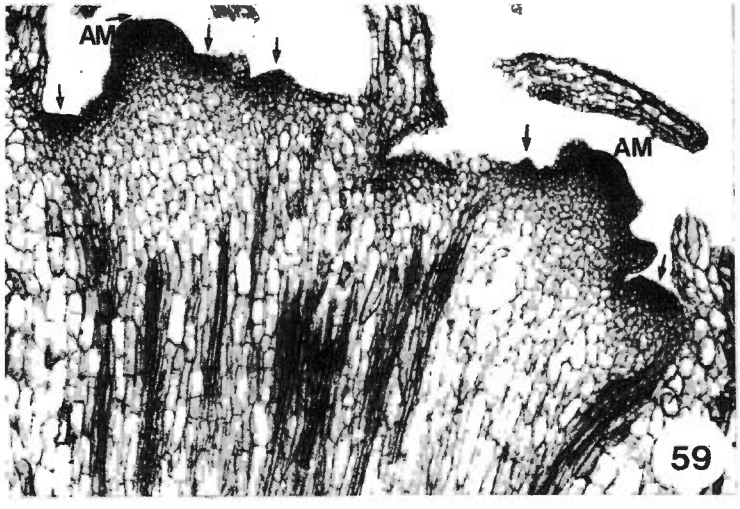
Fig. 61 - Once released from apical dominance the axillary meristems (Ax M) follow the main apical meristem (AM) closely. (LM x 87).

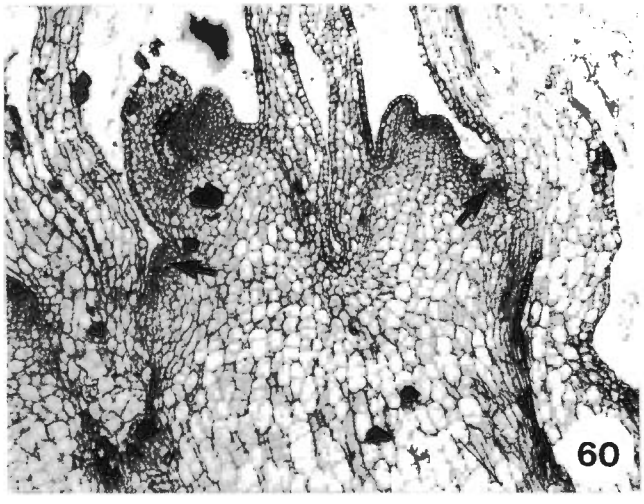
Fig. 62 - Apical part of axillary shoot as in Fig. 61. The apical meristem (AM) is concealed by leaf (L) primordia. Arrows indicate axillary meristems. Initiation of leaf primordium (Lp) occurs on the flank of the axillary meristem in axil of leaf 5. (SEM x 70).

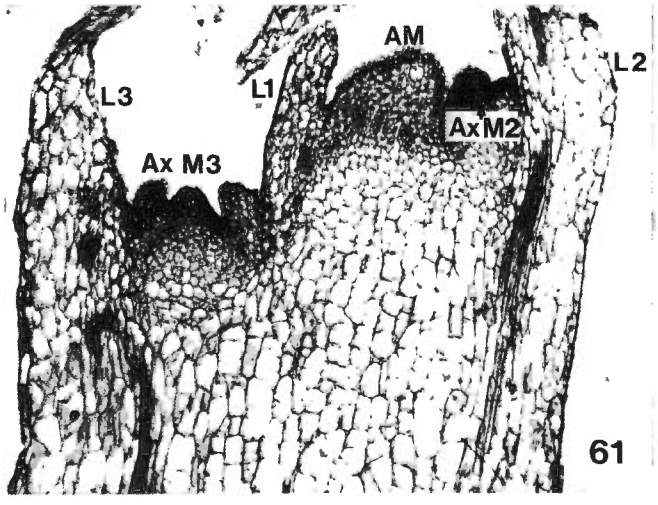












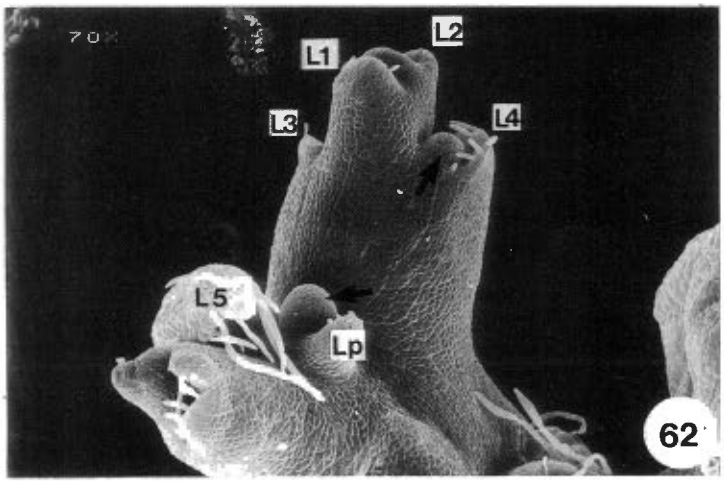


Fig. 63 - Part of dense shoot clump derived from apex of Chenin at 40 days after the start of culture on media enriched with BAP, singly at 2 mg/ℓ as well as in combination with 2 mg/ℓ ZR. Arrows indicate proliferating shoots. (LM x 35).

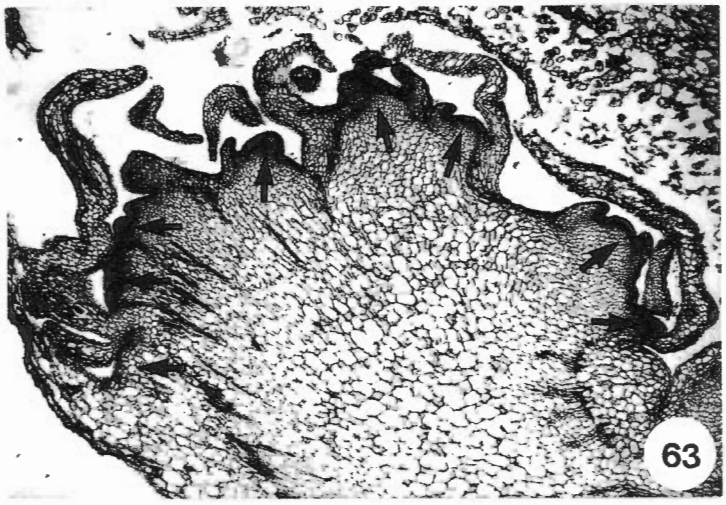
Fig. 64 - Marked elongation of shoots derived from apices of Chenin after 20 days' culture on media enriched with 10 mg/ℓ ZR. Origination of axillary meristems (Ax M) in leaf (L) axils occurs at lower levels down the axes. Note absence of tendrils. (LM x 40).

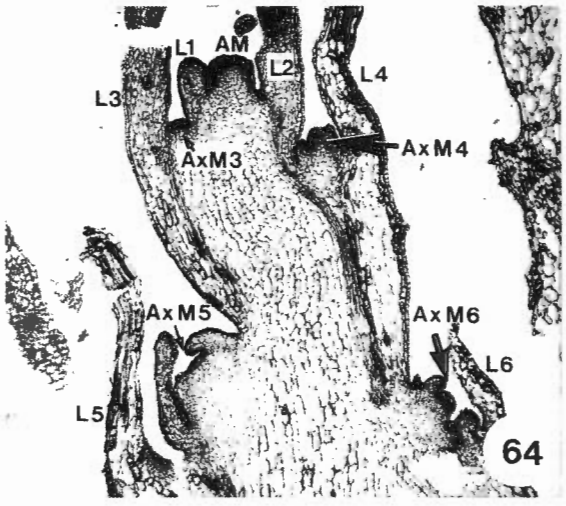
Fig. 65 - Shoot apex after 25 days' culture on media enriched with 10 mg/ℓ ZR, showing apical meristem (AM), 2-layered tunica (Tu), corpus (Corp), leaf buttress (Lb), leaf (L) primordia and primary vascularization (arrowed). The youngest axillary meristem (Ax M) differentiated in axil of leaf 3. (LM x 140).

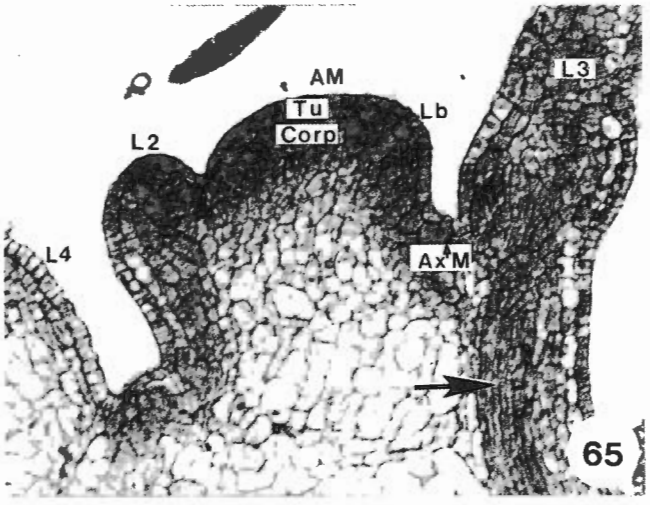
Fig. 66 - Differentiated sieve elements (S) and spirally thickened vessels (V) at base of leaf 3 in Fig. 65. (LM x 220).

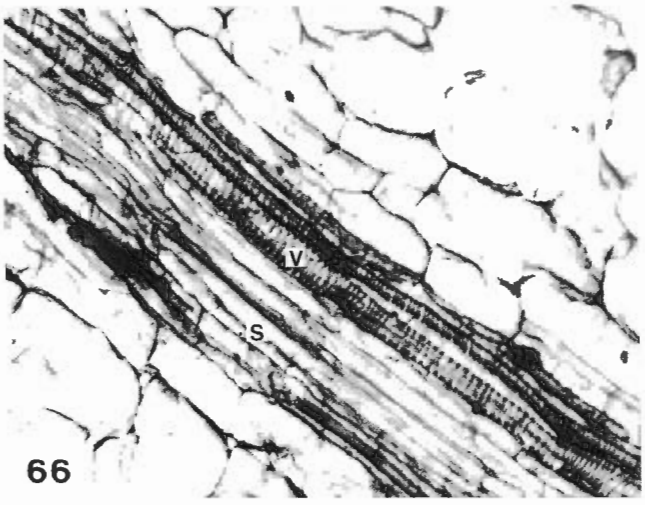
Fig. 67 - Pronounced development and elongation of axillary meristem (Ax M) in axil of leaf 4 after 30 days of culture on media enriched with ZR at 10 mg/ℓ. (LM x 87).

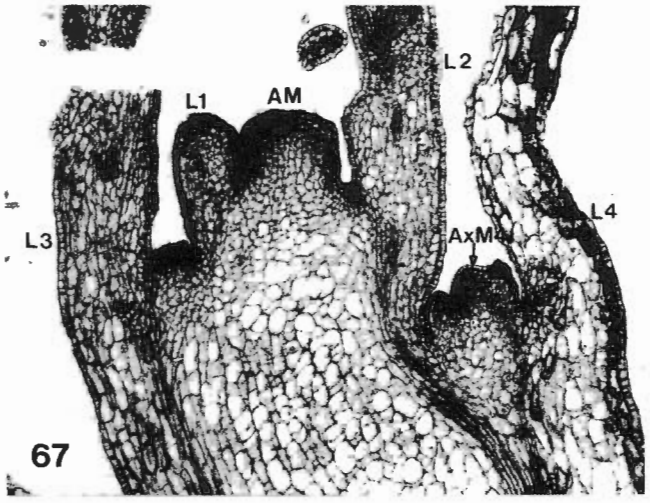
Fig. 68 - Stretching of xylem elements as expressed by the uncoiling of spirals (arrowed) in response to 10 mg/ℓ ZR. (LM x 520).

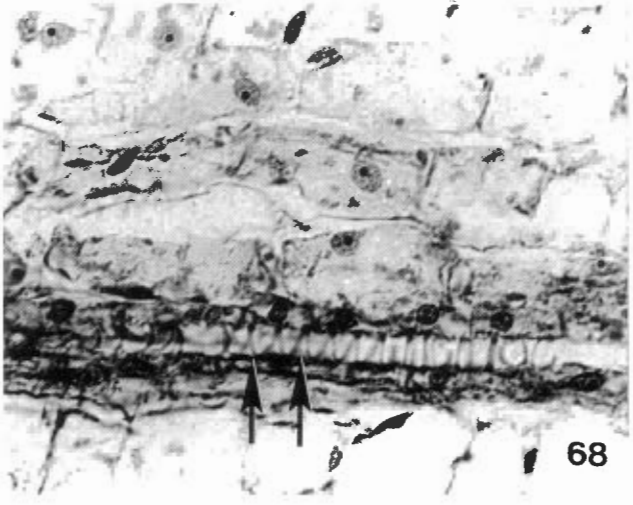












SECTION 6

RESPONSES OF SMALL (≤ 0,2 mm) DETACHED GRAPEVINE SHOOT APICES CULTURED IN VITRO

6.1 Introduction

Successful culturing of small detached shoot apices (< 1 mm) was reported in some woody plant species (Abbott and Whiteley, 1976; Quoirin and Lepoivre, 1977; Tabachnik and Kester, 1977; Chaturvedi et al., 1978). In vitro growth of very small excisions (0,2 mm) of non-fragmented grapevine shoot apices was first described by Aldwinckle and Buturac (1980). The latter report, however, did not indicate whether initial explant growth was dependent on the presence of leaf primordia and whether discolouration (browning) of such small excisions was experienced on the high salt (RM-62) medium of Murashige and Skoog (1962).

It was shown (sections 4 and 5) that growth responses of excised apices (0,7 - 1 mm) cultured in vitro was enhanced by ZR. This experiment was conducted to investigate the effect of explant size (including excisions < 0,2 mm) on successful culturing.

6.2 Procedure

Unless otherwise stated, details of procedure were as outlined earlier (3.1, 3.2, 3.3) using Chenin.

Hypodermic needles were used as microscalpels for the isolation

of explants. Excisions of shoot apices included the following: (1) apical dome excluding adjacent leaf or tendril primordia (0,1 - 0,2 mm); (2) apical dome plus the first defineable leaf primordium (0,2 mm); (3) apical dome plus 1 - 2 leaf primordia (0,5 mm), and (4) apical dome plus 2 - 3 leaf primordia (0,7 - 0,8 mm). Excised apices (1 mm) containing 2 - 4 leaf primordia were used as the control (5). Cultures were treated with 10 mg/ℓ ZR.

A thin layer of sterile distilled water (1 mℓ) was added as a standard procedure to the surface of the solidified medium in each tube after it was observed in preliminary experiments that browning of small excisions (0,1 - 0,2 mm) could be successfully eliminated with such a manipulation. On transfer to the medium the needle carrying the explant was dipped into the water, leaving the latter adrift (Fig. 69).

Individual isolated apex sizes included three replications of 10 explants each and the experiment was terminated after 55 days. Recordings were made of (a) percentages of explants that responded with growth; (b) shoot elongation (length of primary shoots) and (c) shoot proliferation (number of newly formed axillary shoots exceeding 3 mm in length). Data were subjected to analysis of variance followed by Duncan's multiple range test (Snedecor and Cochran, 1967).

6.3 Results and Discussions

Larger explants (0,5 - 1 mm) responded with marked shoot elonga-

tion at 12 days after the start of culture. For smaller explants (0,1 - 0,2 mm) growth proceeded at a much slower rate. Pronounced enlargement occurred after 30 days of culture (Fig. 70). Once commenced, elongation proceeded rapidly, irrespective of initial explant size (Figs. 71, 72). However, elongation slowed down slightly upon the release of axillary shoots from apical dominance. This was especially discernible with larger explants. The layer of fluid, which evaporated after 20 - 25 days of culture, presented no harmful effects.

The effect of explant size on growth percentage is presented in Fig. 73. Lowest percentages were recorded for explants containing apical domes only. Increases in explant sizes (up to 0,2 mm) resulted in a significant rise in growth percentages. A small number (10%) of larger explants (0,5 mm) did not respond with active growth. They enlarged to about twice their original sizes within 35 days and then ceased in further growth. Growth occurred in all cultures (100%) containing initial explants measuring 0,7 - 0,8 mm and 1 mm.

Primary shoot elongation was influenced by initial explant sizes (Fig. 74a). Maximum elongation resulted in explants that contained two and more leaf primordia. Highest numbers of proliferated shoots (> 3 mm) were achieved with explants of 1 mm (Fig. 74b). Although proliferation rates decreased in culturing smaller explants (0,5 - 0,7 mm), they were not significantly lower than those recorded for the control. Lower growth percentages and an initial delay in primary shoot elongation ac-

counted for significantly lower shoot proliferation of small excisions of shoot apices (0,1 - 0,2 mm). Although culturing explants containing apical domes only resulted in limited shoot elongation and proliferation, even after 55 days, the results clearly indicate that initial growth was not entirely dependent on the presence of leaf primordia.

Aldwinckle and Buturac (1980) reported growth of excised apices (0,2 mm) with combined applications of BAP and NAA. After 100 days of culture that included several transfers to fresh media, shoots, 3 - 4 mm in length, were produced. Numbers of newly formed shoots were not indicated. In the present investigation ZR appeared much more effective in stimulating growth (shoot elongation, proliferation and growth percentages) of explants with similar dimensions.

Explants (0,1 - 0,5 mm) that failed to grow or exhibited low growth percentages remained greenish in colour, even after 55 days. The layer of water on the agar surface provided a gradual exposure of the delicate tissues to the high salt concentration in the medium, which might have been the main cause for browning and dying of explants in preliminary experiments. Drying out of the medium, with subsequent increases in concentrations of nutrients was also delayed. In addition, such a layer of fluid may facilitate an effective dispersal of toxic metabolic wastes, should the latter be released by the cultured tissues.

The low rates of success in culturing apical domes only may

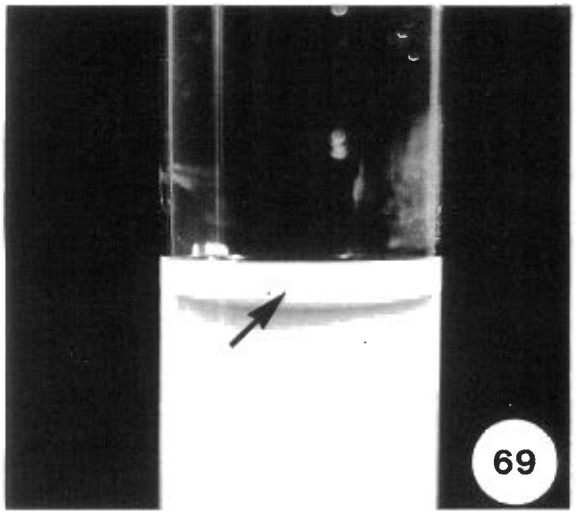
lead to the question whether it would be worthwhile to start a propagation system with such small explants. Due to the potential of cytokinins in enhancing axillary shoot production in subsequent transfers to fresh media it is concluded that such a technique is of considerable practical value.

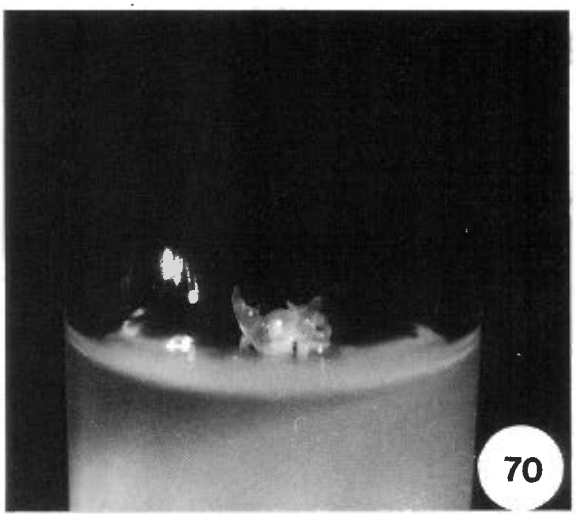
Fig. 69 - Thin layer of sterile distilled water added to the surface of the solidified medium (arrowed). On transfer to the medium explants remained adrift.

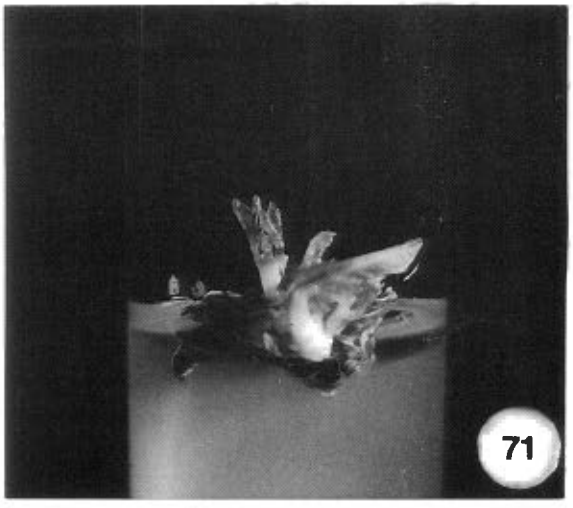
Fig. 70 - Explant growth after 30 days of culture with 10 mg/ℓ ZR.

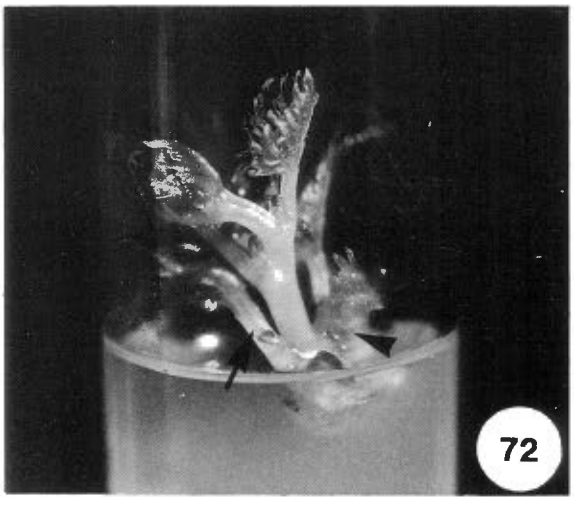
Fig. 71 - Primary shoot elongation at 45 days after the start of culture.

Fig. 72 - Shoot elongation at 55 days after the start of culture. Arrows indicate axillary shoot growth.









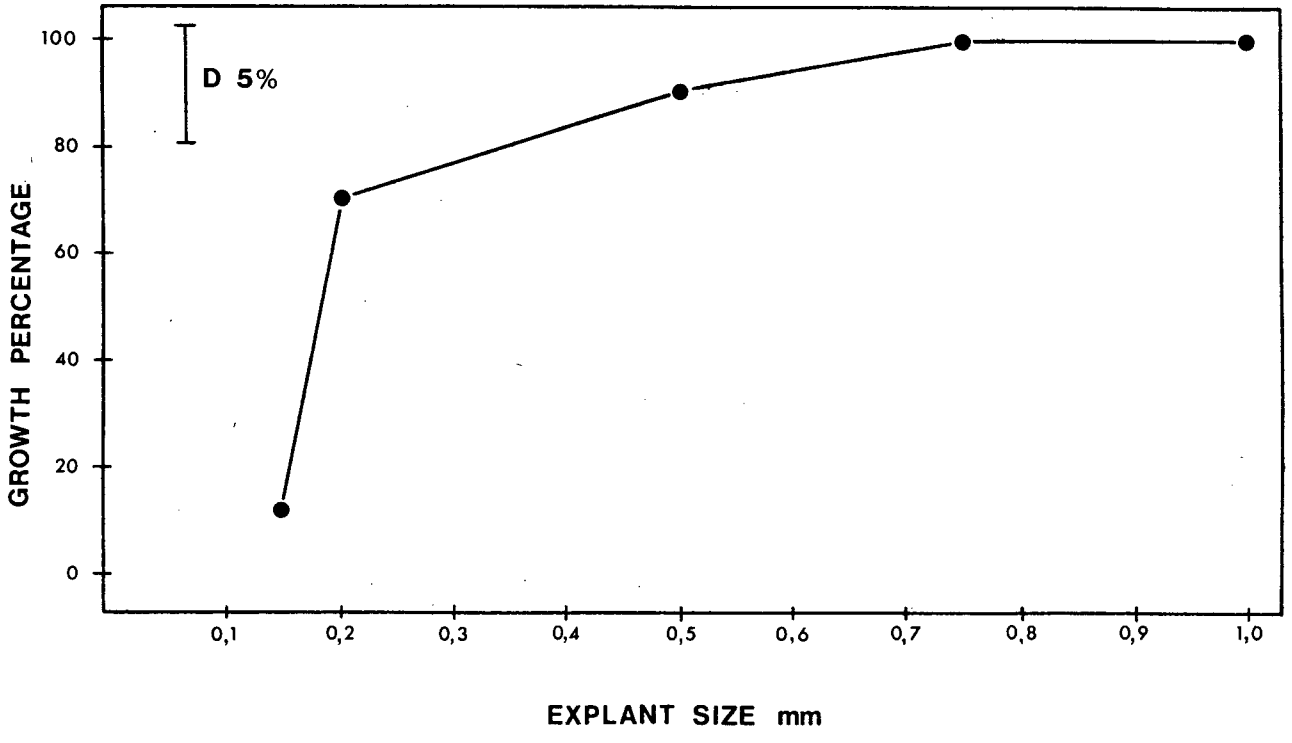


Fig. 73 - The influence of explant size (lengths in mm) on growth percentage (percentage of explants showing growth) in Chenin. Culture period = 55 days.

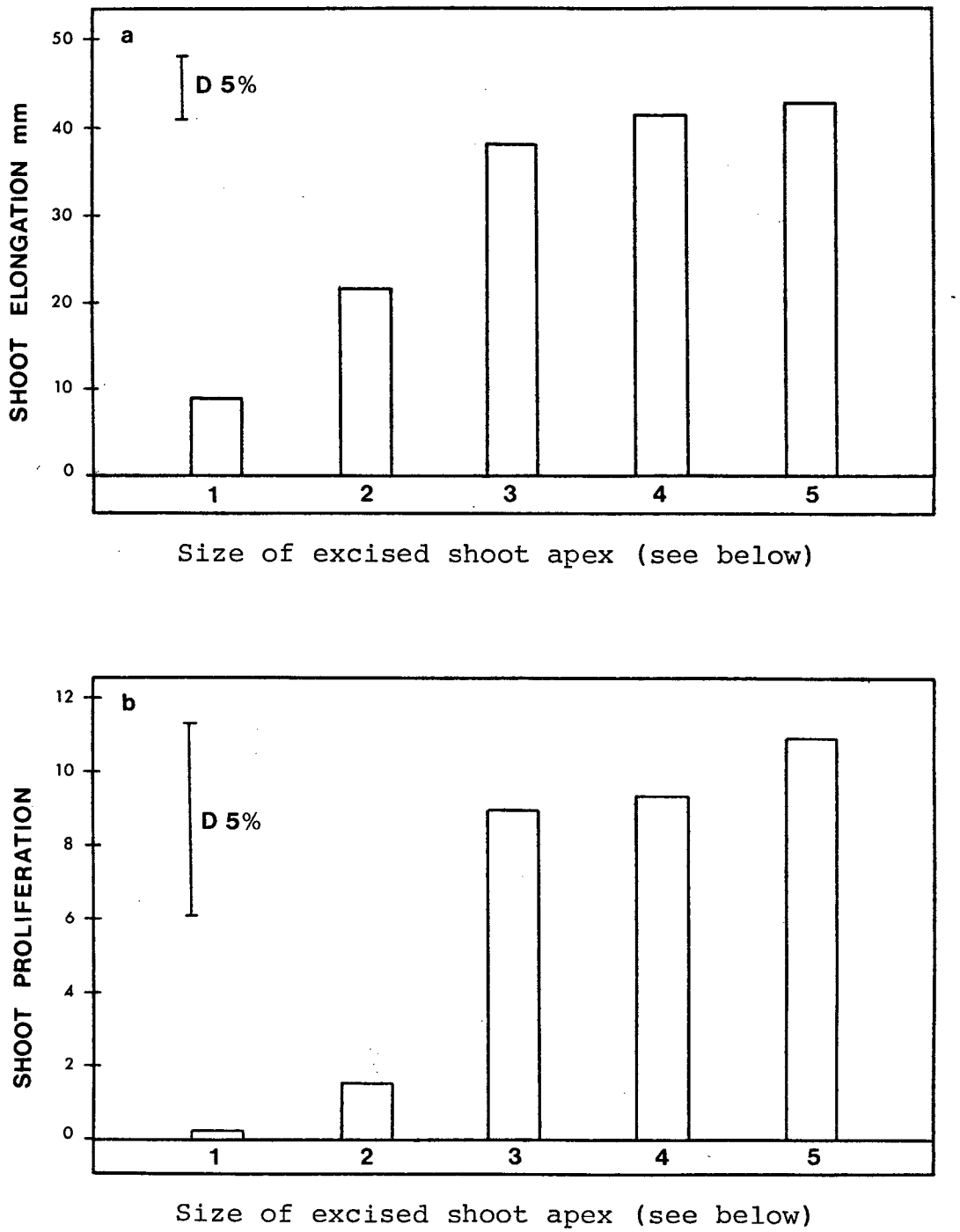


Fig. 74 - In vitro elongation of primary shoots (a) and number of newly formed axillary shoots (b) with 10 mg/ℓ ZR from excised shoot apices of Chenin measuring 0,1 - 0,2 mm (1); 0,2 mm (2); 0,5 mm (3); 0,7 - 0,8 mm (4), and 1 mm (5). Culture period = 55 days.

SECTION 7

INACTIVATION OF GRAPEVINE FANLEAF VIRUS DISEASE (GFLV) BY HEAT TREATMENT OF APICAL REGIONS OF RAPIDLY ELONGATING SHOOTS OF POTTED PLANTS ONLY AND OF SHOOTS WITHOUT ROOTS GROWN IN VITRO

7.1 Introduction

The most common virus inactivation procedures comprise heat treatment of entire potted vines, after which young shoot tips are propagated (Goheen *et al.*, 1965; Nyland and Goheen, 1969; Goheen and Luhn, 1973; Bovey, 1980; Stellmach, 1980a,b). Reports are lacking on the application of heat treatment to certain regions only of virus-infected vines. Experiment A of this section was conducted to investigate the inactivation rate of GFLV by the application of heat to apical regions of elongating shoots only.

In employing fragmented shoot apex culture, Barlass *et al.* (1982) obtained plants cured from the graft-transmissible diseases leaf-roll, yellow speckle, fleck and summer mottle. The latter technique, in combination with heat therapy of the cultures, also proved effective for the inactivation of GFLV (Barlass *et al.*, 1982). Experiment B of this section was conducted to investigate the effect of heat treatment during *in vitro* culture of non-fragmented shoot apices on growth responses and GFLV inactivation.

7.2 Procedure

7.2.1 Source of infected material

Material was sampled from a V. rupestris cv. Rupestris du Lot vine, showing typical fanleaf symptoms.

7.2.2 Heat chamber

All treatments were conducted in a transfer chamber as described previously (3.6.2). Heat was provided by thermostatically controlled incandescent lamps. The temperature within the chamber was maintained at 37 - 40°C during the day and 22 - 25°C during the night.

7.2.3 Detection of GFLV

An Enzyme-Linked Immunosorbent Assay (ELISA) test in accordance with the procedure outlined by Clark and Adams (1977) was applied to detect GFLV in leaf and shoot extracts.

One hour after adding the substrate (p-nitrophenyl phosphate) the photometric absorbance of each well was read at 405 nm with a Titertek Multiscan from Flow. Readings were judged to be positive if the average A405 of a test sample exceeded the A405 of the healthy control sample in the same test plate by a factor of two or more (Voller et al., 1977). In each plate tests were replicated five times. Healthy and GFLV-infected grapevine leaf samples and buffer controls were included in each plate.

7.2.4 Experiment A

The establishment and maintenance of infected vines were described

earlier (3.6.1). At least 50 actively growing vines showing severe fanleaf symptoms, were selected. From these, shoot tips (15 mm), middle and basal leaves were sampled at random and tested with ELISA. Candidate vines for heat therapy were placed underneath the chamber and the elongating shoots guided through the holes in the perspex to reach the inside (Fig. 1).

After 30 days of heat treatment test samples (same as those prior to heat treatment) were removed from shoot regions inside the chamber and subjected to the ELISA test. The shoots were afterwards cut off at 10 cm above the perspex bottom. This procedure was repeated after 60, 90 and 120 days. At each of these intervals (starting at 30 days) newly developed leaves that had received no heat treatment (those below the chamber) were also subjected to ELISA. Throughout the duration of treatments, root systems were at room temperature (20 - 25°C).

7.2.5 Experiment B

The procedure for the activation of shoot primordia from dormant canes and excision of shoot apices (0,5 mm) were outlined earlier (3.1). Prior to heat treatment, explants were cultured in the presence of 10 mg/ℓ ZR under conditions as described previously (3.2, 3.3). After 40 days cultures were removed from test tubes, shoot apices (0,5 mm) excised and the remaining clumps tested with ELISA.

Upon excision, apices were transferred to fresh ZR-enriched media and subjected to heat therapy in the chamber under the

following conditions: (1) culturing under ordinary light conditions (fluorescent tubes of low intensity in laboratory) and (2) culturing under dark conditions (complete wrapping of test tubes in tin-foil). A third treatment consisted of initial culturing with 2 mg/ℓ GA_3 for six days after which apices (0,5 mm) were excised again and transferred to the ZR-enriched medium (ordinary light conditions). Each treatment consisted of 10 cultures. This procedure was repeated at 30, 60, 90 and 120 days after the start of heat treatment. At each interval shoot clumps (after apices had been excised) were subjected to the ELISA test.

7.3 Results and Discussion

7.3.1 Experiment A

The results are presented in Fig. 75.

Prior to heat treatment the virus content of samples of shoot tips (15 mm) exceeded that of leaves taken from the middle or basal parts of shoots. Once exposed to heat, shoot elongation proceeded rapidly. After 10 days newly formed leaves were visually free of fanleaf symptoms in contrast to older leaves on the same vine.

After 30 days of heat treatment, shoot tips (15 mm) reacted negatively to the presence of GFLV whilst middle and basal leaves were still ELISA positive. The virus thus eliminated firstly in those parts of the plant that exhibited strong growth

responses while being heat treated. This is in agreement with reports of Stellmach (1980a).

Removal of apical regions of shoots induced vigorous sprouting and growth of lateral shoots. After 60, 90 and 120 days respectively, shoot tips and leaves on these laterals reacted negatively to the presence of GFLV. Throughout the duration of the experiment, untreated leaves (below the perspex) showed typical fanleaf symptoms and tested highly positive with ELISA.

The present investigation showed that high temperatures were effective in inactivating GFLV in treated regions of infected plants. The virus was not inactivated in the whole plant, as shown by ELISA tests for untreated regions.

The construction and maintenance of successfully operating heat chambers (after the model of Kriedemann et al. 1976) are highly expensive and mostly available only to highly sophisticated institutes and nurseries. The system described above was operated for 240 days without deterioration in the growth of vines. Due to its simplicity, low cost and efficiency as regards the inactivation of GFLV, the described system may have a wider application.

7.3.2 Experiment B

The results are summarised in Figs. 76 - 79.

In the presence of ZR, in vitro heat treatment resulted in

rapid growth of GFLV-infected shoot apices (Figs. 76, 77). In previous sections (4, 5 and 6) excised apices responded to ZR with marked shoot elongation and proliferation. However, these results had been achieved under high light intensities (3 500 lux) and at temperature regimes of 25 - 27°C. In the present investigation the above growth responses were stimulated to a high extent by fluctuating temperatures (up to 40°C) irrespective of sub-optimal light intensities. Rapid elongation was especially discernible in etiolated shoots (Fig. 78). Initiation of adventitious buds from fragmented apices was enhanced at a constant high temperature (35°C), whilst at 38°C apical fragments quickly browned and died (Barlass et al., 1981). In the present investigation fluctuating temperatures (up to 40°C) clearly stimulated growth of non-fragmented shoot apices.

Within 30-day intervals shoot clumps responded with submerged callus growth. A compact, nodular callus tissue resulted. Although no roots were initiated, shoot clumps survived to a great extent without any browning or deterioration of tissues.

After 30 and 60 days, proliferated shoots reacted positively to the presence of GFLV with the ELISA test (Fig. 79). The virus content of samples was, however, much lower after 60 than after 30 days. After 90 and 120 days, etiolation as well as initial culturing with GA_3 resulted in complete inactivation of GFLV. In the presence of 10 mg/ℓ ZR at ordinary light conditions the virus was inactivated after 120 but not 90 days. The results clearly indicated that the inactivation rate of GFLV increased

with treatments that primarily stimulated shoot elongation.

Barlass et al. (1982) succeeded in inactivating GFLV from infected vines by culturing fragmented shoot apices at a high temperature (35°C). They also confirmed the presence of fanleaf in the youngest leaf primordia at the apex and suggested that, as culturing at 27°C was unable to inactivate GFLV in apical fragments, its replication appeared to be temperature-dependent. This investigation showed that GFLV was present in non-fragmented shoot apices (0,5 mm) of infected vines. The virus was eliminated by heat treatment (37 - 40°C during the day and 22 - 25°C during the night) of excised apices (0,5 mm) grown in vitro without roots.

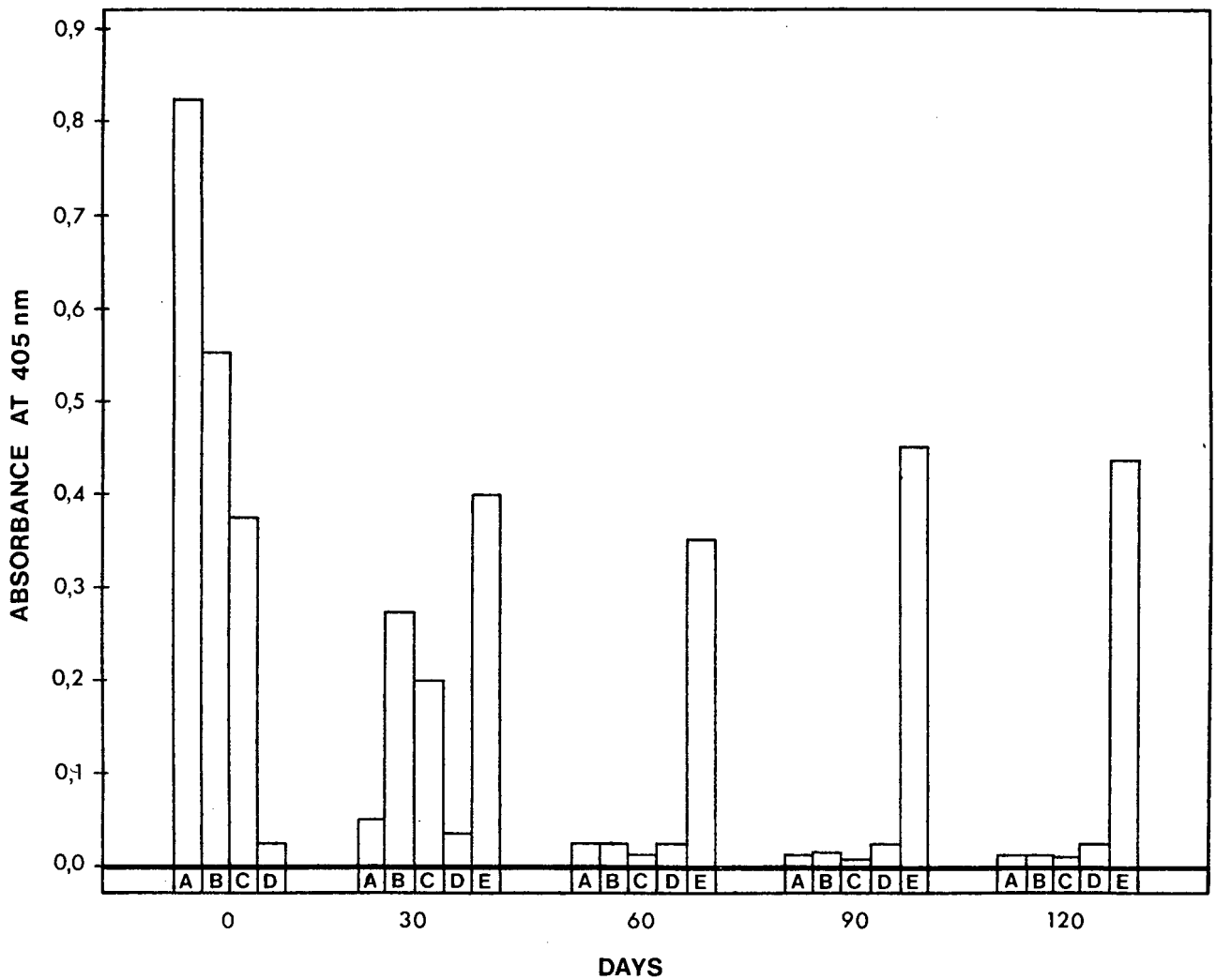
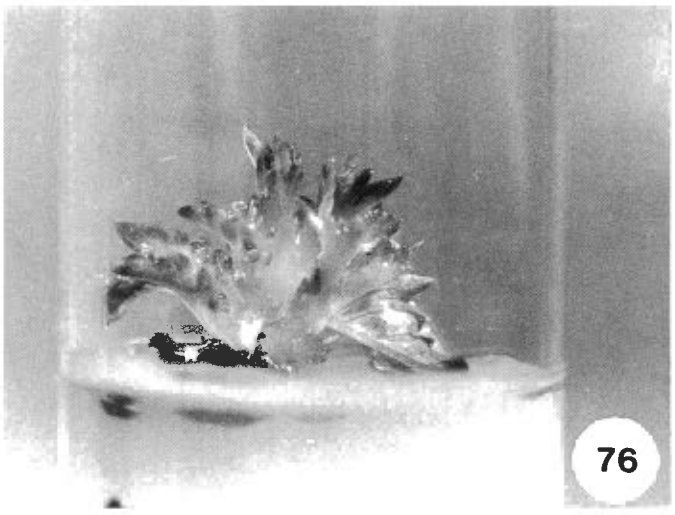


Fig. 75 - Grapevine fanleaf virus (GFLV) concentration in shoot tip and leaf extracts of infected V. rupestris cv. Rup. du Lot after different periods (days) of heat treatment of apical regions of elongating shoots only, as determined by ELISA. A = shoot tips, B = middle leaves, C = basal leaves, D = healthy material (control) and E = untreated leaves.

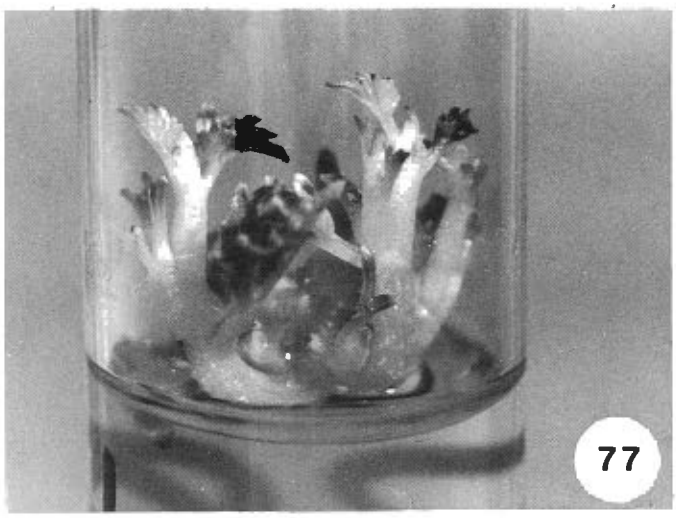
Fig. 76 - Growth of excised shoot apices of GFLV-infected Rup. du Lot during heat treatment *in vitro* with 10 mg/ℓ ZR. Photographed 10 days after transfer to fresh media.

Fig. 77 - Shoot elongation and proliferation of explant in Fig. 76 at 20 days after transfer to fresh media containing 10 mg/ℓ ZR.

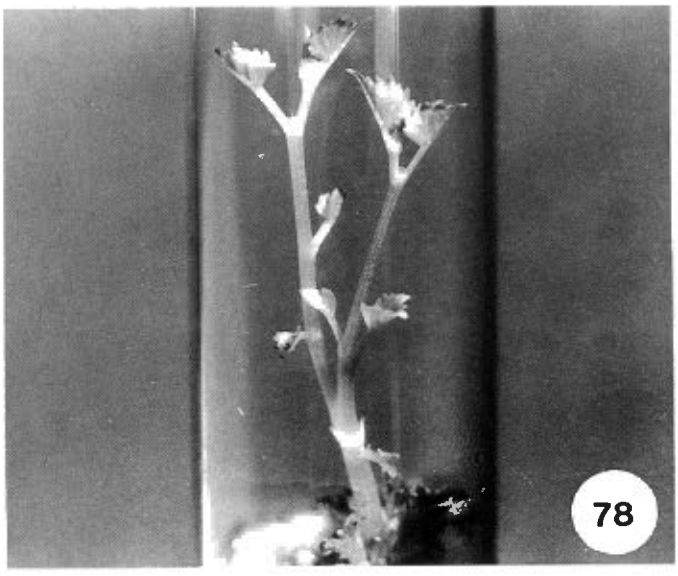
Fig. 78 - Rapid elongation of etiolated shoots during heat treatment *in vitro*. Photographed 20 days after transfer of excised shoot apices to fresh media.



76



77



78

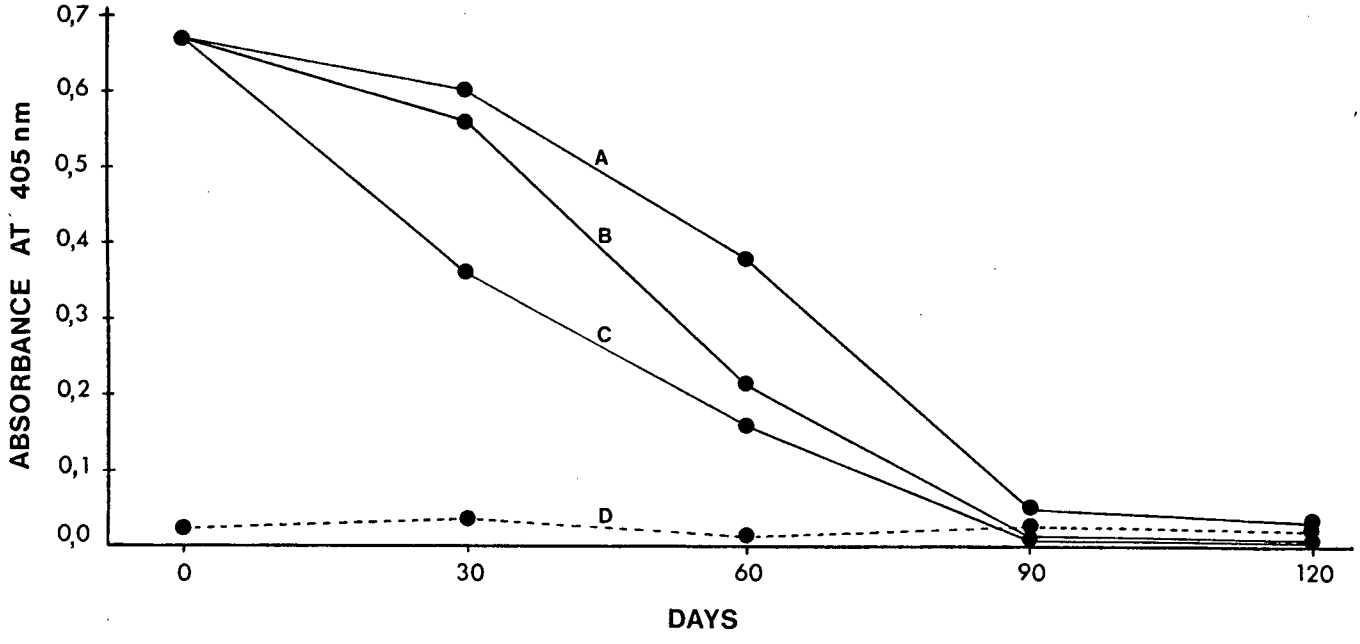


Fig. 79 - Inactivation rate of Grapevine fanleaf virus disease (GFLV) from shoots grown from excised apices of infected Rup. du Lot with 10 mg/ℓ ZR at different periods (days) during heat treatment in vitro, as determined by ELISA. A = ordinary light conditions, B = dark conditions, C = initial culturing with GA_3 followed by transfer to ZR-media (ordinary light conditions) and D = healthy material (control)

SECTION 8

SUMMARY

8.1 Responses of shoot apices of grapevines cultured in vitro

Excised shoot apices (0,75 - 1 mm) of Chenin blanc and of the rootstock cross US 1-9 were cultured *in vitro* on the high salt (RM-62) medium of Murashige and Skoog (1962), amended with myo-inositol, sucrose and various growth substances. Morphological and anatomical responses of explants cultured *in vitro* were investigated.

8.1.1 Effect of cytokinins

Cytokinins were tested at concentrations ranging from 0 - 20 mg/ℓ. Primary shoot elongation, leaf expansion and axillary shoot growth (proliferation) were stimulated by 6-benzylaminopurine (BAP), kinetin, zeatin and zeatin riboside (ZR) although kinetin was significantly less effective. ZR appeared the most favourable for elongation of primary as well as axillary shoots once the latter were released from apical dominance. Although BAP proved the most effective in overcoming of apical dominance, elongation of newly formed shoots was suppressed. This gave rise to the formation of dense shoot clusters bearing atypical leaves. Optimal levels for these effects varied greatly between the different cytokinins.

8.1.2 Effect of cytokinin:auxin combinations

Combined applications of kinetin (0,1 - 8 mg/ℓ) with indole-

3-acetic acid (IAA) (0,5 - 16 mg/ℓ), 1-naphthaleneacetic acid (NAA) (0,1 - 8 mg/ℓ) or indole-3-butyric acid (IBA) (0,1 - 8 mg/ℓ) and of 6-benzylaminopurine (BAP) (0,5 - 3,5 mg/ℓ) or zeatin riboside (ZR) (1 - 10 mg/ℓ) with NAA (0,1 - 5 mg/ℓ) were tested.

Kinetin-induced shoot elongation and leaf expansion were suppressed in decreasing order of activity by NAA, IBA and IAA. However, in combination with the latter auxin, kinetin effects were slightly enhanced provided IAA concentrations were kept at low levels. Stimulatory effects of BAP and ZR on shoot elongation were strongly suppressed by NAA.

Combined applications of kinetin with IAA, NAA or IBA were ineffective in inducing shoot proliferation. Stimulatory effects of BAP on shoot proliferation were suppressed in the presence of NAA. Proliferation rates were, however, stimulated by an interaction between ZR and NAA in the cv. Chenin blanc, provided NAA levels did not exceed 0,1 mg/ℓ.

Of the auxins tested, only NAA gave rise to callus growth at the basal cut surfaces of explants. No callus initiation occurred in the presence of cytokinins alone. In the presence of both cytokinin and auxin, callus growth was greatly affected by the ratio between these two growth substances. However, this did not apply to all treatments as with KIN:IAA combinations no callus growth occurred. Although in the majority of treatments more callus growth occurred with moderate concentrations of cytokinin and auxin in combination than with either high cyto-

kinin/auxin or high auxin/cytokinin ratios, the results varied considerably. In addition, the cv. US 1-9 responded to a much higher degree with callus growth than Chenin blanc to the applied cytokinin:auxin ratios.

Rooting was suppressed in the presence of auxin and cytokinin alone. With combined applications of these growth regulators rooting occurred only in Chenin blanc. Although more roots resulted from the application of lower NAA levels combined with higher ZR concentrations, root elongation was suppressed. Suppressive effects of NAA on root elongation were not overcome by ZR treatments, even at 10 mg/ℓ.

8.1.3 Effect of cytokinin:gibberellin combinations

Combined applications of ZR (1 - 10 mg/ℓ) and gibberellic acid (GA_3) (0,5 - 10 mg/ℓ) were tested. Gibberellin-induced shoot elongation and leaf expansion was stimulated by ZR. Inhibitory effects of GA_3 (even at 0,5 mg/ℓ) on the release of axillary meristems from apical dominance were not alleviated in the presence of ZR (even at 10 mg/ℓ).

8.1.4 Effect of cytokinin:cytokinin combinations

Combined applications of BAP (0,5 - 5 mg/ℓ) with ZR (1 - 10 mg/ℓ) were tested. If supplied separately, maximum shoot elongation and proliferation resulted with BAP at 2 mg/ℓ or with ZR at 10 mg/ℓ. Both processes were inhibited by BAP at 5 mg/ℓ. Addition of ZR (even at 10 mg/ℓ) failed in cancelling this inhibition. Optimal shoot proliferation resulted from application

of these cytokinins in combination, provided BAP levels did not exceed 2 mg/ℓ.

8.1.5 Effect of cytokinins in routine subculturing

Shoots derived from excised apices of Chenin were repeatedly transferred to media containing different concentrations of BAP and ZR. Optimal shoot proliferation over five subcultures resulted with a combination of the cytokinins, each at 2 mg/ℓ. Shoots produced with 10 mg/ℓ ZR alone elongated rapidly, were slim and erect, with almost normal leaves. In the presence of BAP at 2 mg/ℓ alone and in combination with low concentrations of ZR (2 mg/ℓ), proliferated shoots were thicker, often branched, less elongated, curved and not uniform in size. Leaves were atypical. Cytokinin application in routine subculturing proved highly effective in the production of large numbers of grapevine clones.

8.1.6 Anatomical responses

Responses of explants of Chenin blanc to different cytokinin treatments were studied at various intervals (days) after the start of culture, using light and scanning electron microscopy (SEM).

Results clearly indicated that *in vitro* produced shoots were of axillary origin. Shoot clusters were induced by the enhanced release of axillary meristems from apical dominance, due to the application of BAP at 2 mg/ℓ as well as in combination with 2 mg/ℓ ZR. Axillary meristems on these axillary shoots were

subsequently released from apical dominance, thus giving rise to clusters of high densities. Shoot clusters with ZR alone at 10 mg/ℓ were less dense, probably due to an initial delay in elongation of axillary meristems nearest to the main apical meristem of the shoot (AMS). However, elongation of axillary meristems at lower levels down the axes proceeded strongly in the presence of 10 mg/ℓ ZR. Application of BAP resulted in more pronounced release of axillary meristems from apical dominance than with ZR.

8.1.7 Effect of explant size on successful culturing

Small excisions (0,1 - 1 mm) of shoot apices of Chenin blanc were cultured *in vitro* on ZR-enriched media. Growth responses were highly influenced by explant size. Lowest growth percentages resulted with explants containing apical domes only (0,1 mm). The latter excisions were, however, independent of the presence of leaf primordia in responding with primary shoot elongation. In larger explants ($\geq$ 0,2 mm) growth responses were enhanced.

8.2 Inactivation of grapevine fanleaf virus disease (GFLV)

8.2.1 Heat treatment of apical regions of elongating shoots of potted plants only

A transfer chamber, equipped with thermostatically controlled incandescent lamps, was used as heat chamber. Discontinuous heat treatment (37 - 40°C during the day and 22 - 25°C during the night) was applied to apical regions of elongating shoots only of vines of Rupestris du Lot infected with GFLV. Root systems were kept at room temperature. An Enzyme-Linked Immunosorbent

Assay (ELISA) test was used to detect GFLV in material at 30-day intervals after the start of heat treatment.

Heat treatment induced marked elongation of primary as well as lateral shoots inside the chamber. After 30 days, newly formed shoots and leaves reacted negatively to the presence of GFLV with the ELISA test. The system was operated for 240 days without diminished shoot growth. Throughout the duration of treatments leaves on elongating laterals on the outside of the chamber showed typical fanleaf symptoms and tested highly positive with ELISA. The results indicated that high temperatures were effective in the inactivation of GFLV from treated regions of infected vines.

8.2.2 Heat treatment of shoots without roots grown in vitro

Responses of excised shoot apices of Rupestris du Lot infected with GFLV to heat therapy in vitro were investigated. Daily cycles of high (37 - 40°C) and low (22 - 25°C) temperatures were effective in promoting shoot elongation and proliferation in the presence of 10 mg/ℓ ZR. It was demonstrated that discontinuous heat therapy succeeded in the inactivation of GFLV in proliferated shoots. Treatments that induced active shoot elongation of explants were more efficient in inactivating the virus.

REFERENCES

ABBOTT, A.J., 1978. Practice and promise of micropropagation of woody species. Acta Hort. 79, 113 - 127.

ABBOTT, A.J. & WHITELEY, E., 1976. Culture of Malus tissues in vitro. I. Multiplication of apple plants from isolated shoot apices. Sci. Hort. 4, 183 - 189.

ABO EL-NIL, M.M. & HILDEBRANDT, A.C., 1971. Differentiation of virus-symptomless geranium plants from anther callus. Plant Dis. Rep. 55, 1017 - 1020.

ABO EL-NIL, M.M. & HILDEBRANDT, A.C., 1973. Origin of androgenetic callus and haploid geranium plants. Can. J. Bot. 51, 2107 - 2109.

ABO EL-NIL, M.M. & HILDEBRANDT, A.C., 1974. Tobacco ringspot virus transmission and symptoms on seedlings of anther cultured geraniums. In vitro 9, 362.

ADAMS, A.N., 1972. An improved medium for strawberry meristem culture. J. Hort. Sci. 47, 263 - 264.

ADAMS, A.N., 1975. Elimination of viruses from the hop (Humulus lupulus) by heat therapy and meristem culture. J. Hort. Sci. 50, 151 - 160.

ALDWINCKLE, H.S. & BUTURAC, I., 1980. Culture of grape cultivars from apical meristems. Proc. 7th meeting ICVG, Niagara Falls, Canada, pp. 339 - 341.

ALLAN, P., 1981a. Plant propagation through tissue culture. The S.A. Nurseryman, February/March, 31 - 39.

ALLAN, P., 1981b. Plant propagation through tissue culture. The S.A. Nurseryman, May/June, 5 - 22.

ALPHI, A., 1965. Coltura degli apici vegetativi di garofano (Dianthus caryophyllus) e di orchidea (Cymbidium sp.). Rivista dell' Ortofloro-frutticoltura Italiana Anno 90. Vol. XLIX, 460 - 466.

ANDERSON, W.C., 1975. Propagation of rhododendrons by tissue culture. Part. I. Development of a culture medium for multiplication of shoots. Proc. Inter. Plant Prop. Soc. 25, 129 - 135.

ANDERSON, W.C., 1980. Mass propagation by tissue culture: Principles and techniques. Proceedings of the Conference on Nursery Production of Fruit Plants through Tissue Culture - Applications and Feasibility, Beltsville, Maryland, pp. 1 - 10.

APPIANO, A. & PENNAZIO, S., 1972. Electron microscopy of potato meristem tips infected with potato virus X. J. Gen. Virol. 14, 273 - 276.

BAJAJ, Y.P.S. & DIONNE, L.A., 1966. Virus X free roots from infected potato plants. Amer. Potato J. 43, 384.

BAKER, K.F., 1962. Thermotherapy of planting material. Phytopathology 52, 1244 - 1255.

BAKER, R. & PHILLIPS, D.J., 1962. Obtaining pathogen-free stock by shoot tip culture. Phytopathology 52, 1242 - 1244.

BALL, E., 1941. The development of the shoot apex and of the primary thickening meristem in Phoenix canariensis Chaub., with comparisons to Washingtonia filifera Wats. and Trachycarpus excelsa Wendl. Am. J. Bot. 28, 820 - 832.

BARLASS, M. & SKENE, K.G.M., 1978. In vitro propagation of grapevine (Vitis Vinifera L.) from fragmented shoot apices. Vitis 17, 335 - 340.

BARLASS, M. & SKENE, K.G.M., 1979. Clonal propagation of vines through tissue culture. Austral. Grapegrower and Winemaker 16(191), 12 - 13.

BARLASS, M. & SKENE, K.G.M., 1980. Studies on the fragmented shoot apex of grapevine. II. Factors affecting growth and differentiation *in vitro*. J. Exp. Bot. 31, 489 - 495.

BARLASS, M., SKENE, K.G.M. & CLINGELEFFER, P.R., 1981. Studies on the fragmented shoot apex of grapevine. III. A scanning electron microscope study of adventitious bud formation *in vitro*. J. Exp. Bot. 32, 1079 - 1083.

BARLASS, M., SKENE, K.G.M., WOODHAM, R.C. & KRAKE, L.R., 1982. Regeneration of virus-free grapevines using *in vitro* apical culture. Ann. Appl. Biol. 101, 291 - 295.

BELKENGREN, R.O. & MILLER, P.W., 1962. Culture of apical meristems of *Fragaria vesca* strawberry plants as a method of excluding latent A virus. Plant Dis. Rep. 46, 119 - 121.

BEN-JAACOV, J. & LANGHANS, R.W., 1972. Rapid multiplication of chrysanthenum plants by stem-tip proliferation. Hort. Sci. 7, 289 - 290.

BERBEE, F.M., BERBEE, J.G. & HILDEBRANDT, A.C., 1972. Induction of callus and trees from stem-tip cultures of a hybrid poplar. *In vitro* 7, 269.

BERBEE, F.M., BERBEE, J.G. & HILDEBRANDT, A.C., 1973. Induction of callus and virus symptomless plants from stem-tip cultures of cassava. *In vitro* 8, 421.

BERTHELOT, A., 1934. Nouvelles remarques d'ordre chimique sur le choix des milieux de culture naturels et sur la maniére de formuler les milieux synthetiques. Bull. Soc. Chim. Biol. Paris 16, 1553 - 1557.

BINI, G., 1976. Prove di cultura "in vitro" di meristemi apicalli di Vitis vinifera L. Riv. Ortoflorofruttic. Ital. 60, 289 - 296.

BOVEY, R., 1980. Control of virus and virus-like diseases of grapevine: Sanitary selection and certification, heat therapy, soil fumigation and performance of virus-tested material. Proc. 7th meeting ICVG, Niagara Falls, Canada, pp. 299 - 309.

BOXUS, Ph., 1974. The production of strawberry plants by in vitro micro-propagation. J. Hort. Sci. 49, 209 - 210.

BOXUS, Ph., 1975. La culture de méristémes de Prunus pour l'obtention de plants sains. Acta Hort. 44, 43 - 46.

BOXUS, Ph., 1976. Rapid production of virus-free strawberry by in vitro culture. Acta Hort. 66, 35 - 38.

BRANAS, J., 1957. Sur quelques données ontogéniques. Prog. Agr. Vitic. 148, 58 - 67.

BRANTS, D.H., 1968. A revised medium for Freesia meristem cultures. Neth. J. Plant Path. 74, 120 - 121.

BROADBENT, L., 1964. Control of virus diseases. In: Plant Virology, pp. 330 - 364. Eds. M.K. Corbett and H.D. Sisler. Gainesville: Univ. of Florida Press.

BROOME, O.C. & ZIMMERMAN, R.H., 1978. In vitro propagation of blackberry. Hort. Sci. 13, 151 - 153.

BRüCKBAUER, H., 1979. Untersuchungen zur Thermotherapie der Reben. Verhalten von Rebsorten bei hohen Temperaturen. Wein-Wiss. 34, 264 - 278.

BUTTROSE, M.S., 1969. Vegetative growth of grapevine varieties under controlled temperature and light intensity. Vitis 8, 280 - 285.

BUVAT, R., 1952. Structure, évolution et fonctionnement du méristème apical de quelques dicotylédones. Ann. des Sciences Nat. Bot. XIII, 199 - 300.

BUVAT, R., 1955. Le méristème apical de la tige. Ann. Biol. 31, 595 - 656.

CHAMBERS, J., 1961. The production of virus-free raspberry plants. J. Hort. Sci. 36, 48 - 54.

CHANDRA, N. & HILDEBRANDT, A.C., 1967. Differentiation of plants from tobacco mosaic virus inclusion-bearing and inclusion-free single tobacco cells. Virology 31, 414 - 421.

CHATURVEDI, H.C., SHARMA, A.K. & PRASAD, R.N., 1978. Shoot apex culture of Bougainvillea glabra "Magnifica". Hort. Sci. 13, 36.

CHÉE, R. & POOL, R.M., 1982. The effects of growth substances and photoperiod on the development of shoot apices of Vitis cultured in vitro. Sci. Hort. 16, 17 - 27.

CHENG, T.-Y., 1978. Clonal propagation of vines through tissue culture techniques. Proc. Inter. Plant Prop. Soc. 28, 139 - 155.

CHENG, T.-Y., 1979. Micropropagation of clonal fruit tree root-stocks. Compact Fruit Tree 12, 127 - 137.

CHRISTENSEN, M., 1968. Eliminering af kartoffel virus X i kartoffelsorten Dianella. Tidsskr. Planteavl. 72, 241 - 244.

CHUTTER, M.E., 1960. Hormonal induction of vascular tissue in tobacco pith in vitro. Science 132, 548 - 549.

CLARK, M.F. & ADAMS, A.N., 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34, 475 - 483.

CLOWES, F.A.L., 1961. Apical meristems. Botanical Monographs Vol. 2. Oxford, Blackwell.

COHEN, D., 1977. Thermotherapy and meristem-tip culture of some ornamental plants. Acta Hort. 78, 381 - 388.

COLIN, J. & VERHOYEN, M., 1976. Anatomical study of Prunus meristematic tips. Acta Hort. 67, 87 - 95.

DEBERGH, P. & MAENE, L., 1977. Rapid clonal propagation of pathogen-free Pelargonium plants starting from shoot tips and apical meristems. Acta Hort. 78, 449 - 454.

DE FOSSARD, R.A., 1976. Tissue culture for plant propagators. Armidale: Univ. of New England Printing.

DE VOS, M.P., 1974. B. Honneurs: Mikrotegniek (roneoed notes).

EARLE, E.D. & LANGHANS, R.W., 1974. Propagation of chrysanthemum in vitro. I. Multiple plantlets from shoot tips and the establishment of tissue cultures. J. Am. Soc. Hort. Sci. 99, 128 - 132.

EARLE, E.D. & LANGHANS, R.W., 1975. Carnation propagation from shoot tips cultured in liquid medium. Hort. Sci. 10, 608 - 610.

ELLIOT, R.F., 1970. Axenic culture of meristem tips of Rosa multiflora. Planta 95, 183 - 186.

ESAU, K., 1942. Vascular differentiation in the vegetative shoot of Linum. I. The procambium. Am. J. Bot. 29, 738 - 747.

ESAU, K., 1943a. Vascular differentiation in the vegetative shoot of Linum. II. The first phloem and xylem. Am. J. Bot. 30, 248 - 255.

ESAU, K., 1943b. Origin and development of the primary vascular tissues in seed plants. Bot. Rev. 9, 125 - 206.

ESAU, K., 1945. Vascularization of the vegetative shoots of Helianthus and Sambucus. Am. J. Bot. 32, 18 - 29.

FOSTER, A.S., 1939. Problems of structure, growth and evolution in the shoot apex of seed plants. Bot. Rev. 5, 454 - 470.

FOSTER, A.S., 1941. Comparative studies on the structure of the shoot apex in seed plants. Torrey Bot. Club Bul. 68, 339 - 350.

FOURNIOUX, J.C., 1972. Distribution et différenciation des tissus conducteurs primaires dans les organes aeriens de Vitis vinifera L. Ph.D. Thesis, Univ. Dijon.

FULTON, J.P. & McGREW, J.R., 1970. Virus and virus-like diseases of strawberry. In: Virus diseases of small fruits and grapevines, pp. 1 - 75. Ed. N.W. Frazier. Berkeley: Univ. of Calif., Div. of Agric.

GALZY, R., 1964. Technique de thermothérapie des viroses de la Vigne. Ann. des Épiphyties 15, 245 - 256.

GALZY, R., 1972. La culture in vitro des apex de Vitis rupestris. C.R. Acad. Sci. Paris 274, 210 - 213.

GAMBORG, O.L., MURASHIGE, T., THORPE, T.A. & VASIL, I.K., 1976. Plant tissue culture media. In vitro 12, 473 - 478.

GARRISON, R., 1949. Origin and development of axillary buds: Syringa vulgaris. Am. J. Bot. 36, 205 - 213.

GIFFORD, E.M., 1950. The structure and development of the shoot apex in certain woody Ranales. Am. J. Bot. 37, 595 - 611.

GIFFORD, E.M., 1954. The shoot apex in angiosperms. Bot. Rev. 20, 477 - 529.

GIFFORD, E.M. & HEWITT, W.B., 1961. The use of heat therapy and in vitro shoot tip culture to eliminate fanleaf virus from the grapevine. Am. J. Enol. Vitic. 12, 129 - 130.

GOHEEN, A.C. & LUHN, C.F., 1973. Heat inactivation of viruses in grapevines. Riv. di Pat. Veg. 9, 287 - 289.

GOHEEN, A.C. & NYLAND, G., 1971. The big breakthrough: Mist propagation. Wines and Vines 52(5), 25 - 27.

GOHEEN, A.C., LUHN, C.F. & HEWITT, W.B., 1965. Inactivation of grapevine viruses in vivo. Proc. Int. Conf. Virus Vector Perennial Hosts, Davis, California, pp. 255 - 265.

GONSALVES, D., 1982. Reaction of grape varieties to tomato ringspot virus. In: Development in Industrial Microbiology, Vol. 23, pp. 91 - 98. Arlington, Virginia: American Institute of Biological Sciences.

GRENAN, S., 1979. Rhizogenese de bourgeons apicaux de boutures de vigne cultivées in vitro. Conn. de la Vigne et du Vin 13, 125 - 136.

GRINBLAT, U.H., 1972. Differentiation of Citrus stem in vitro. J. Amer. Soc. Hort. Sci. 97, 599 - 603.

GUNCKEL, J.E. & WETMORE, R.H., 1946. Studies of development in long shoots and short shoots of Ginkgo biloba L. I. The origin and pattern of development of the cortex, pith and procambium. Am. J. Bot. 33, 285 - 295.

HACKETT, W.P., 1966. Applications of tissue culture to plant propagation. Proc. Int. Plant Prop. Soc. 16, 88 - 92.

HACKETT, W.P. & ANDERSON, J.M., 1967. Aseptic multiplication and maintenance of differentiated carnation shoot tissue derived from shoot apices. Proc. Amer. Soc. Hort. Sci. 90, 365 - 369.

HAISSIG, B.E., 1965. Organ formation in vitro as applicable to forest tree propagation. Bot. Rev. 31, 609 - 626.

HAMMERSCHLAG, F., 1980. Peach micropropagation. Proceedings of the Conference on Nursery Production of Fruit Plants through Tissue Culture - Applications and Feasibility, Beltsville, Maryland, pp. 48 - 52.

HARRIS, R.E. & STEVENSON, J.H., 1982. In vitro propagation of Vitis. Vitis 21, 22 - 32.

HASEGAWA, P.M., MURASHIGE, T. & TAKATORI, F.H., 1973. Propagation of asparagus through shoot apex culture. II. Light and temperature requirements. Transplantability of plants and cytohistological characteristics. J. Amer. Soc. Hort. Sci. 98, 143 - 148.

HAYAT, M.A., 1974. Principles and Techniques of Scanning Electron Microscopy. New York: Van Nostrand Reinhold Company.

HEGEDüS, A., 1957. Histogenetische Untersuchungen am Sprossvegetationskegel der Weinrebe. (English Summary.) Magy. Tud. Akad. Biol. Közlem. 7, 257 - 275.

HEGEDüS, A., 1969. Deckhaartypen des Rebenblattes. (English Summary.) Magy. Tud. Akad. Biol. Közlem. 15, 25 - 35.

HEINZ, D.J., MEE, G.W.P. & NICKELL, L.G., 1969. Chromosome numbers of some Saccharum species hybrids and their cell suspension cultures. Am. J. Bot. 56, 450 - 456.

HELLER, R., 1953. Recherches sur la nutrition minérale des tissus végétaux cultivés in vitro. Ann. Sci. Nat. Bot. Biol. Végét. 14, 1 - 22.

HILL, G.O., 1968. Shoot formation in tissue cultures of chrysanthenum "Bronze Pride". Physiol. Plant. 21, 386 - 389.

HOEFFER, L.L. & GIFFORD, E.M., 1964. Growth in vitro of excised stem tips of V. vinifera. Am. J. Bot. 51, 677 - 679.

HOLLINGS, M., 1962. Heat treatment in the production of virus-free ornamental plants. N.A.A.S. Quart. Rev. 57, 31 - 34.

HOLLINGS, M. & STONE, O.M., 1964. Investigation of carnation viruses. I. Carnation mottle. Ann. Appl. Biol. 53, 103 - 118.

HOLLINGS, M. & STONE, O.M., 1968. Techniques and problems in the production of virus-tested planting material. Sci. Hort. 20, 57 - 72.

HOLLINGS, M. & STONE, O.M., 1970. Attempts to eliminate chrysanthenum stunt from chrysanthenum by meristem-tip culture after heat treatment. Ann. Appl. Biol. 65, 311 - 315.

HOLMES, F.O., 1960. Rose mosaic cured by heat treatments. Plant Dis. Rep. 44, 46 - 47.

HUSSEY, G., 1977. In vitro propagation of some members of the Lilliaceae, Iridaceae and Amaryllidaceae. Acta Hort. 78, 303 - 309.

HUSSEY, G., 1978. The application of tissue culture to the vegetative propagation of plants. Sci. Progr. Oxford 65, 185 - 208.

HUSSEY, G., 1980. In vitro propagation. In: Tissue Culture Methods for Plant Pathologists, pp. 51 - 61. Eds. D.S. Ingram and J.P. Helgeson. Oxford: Blackwell Scientific Publications.

IZVORSKA, N.D. & RANGELOVA, E.M., 1974. Growing carnation plants from meristem tissues. C.R. Acad. Bulgare Sci. 27, 1581 - 1584.

JABLONSKI, J.R. & SKOOG, F., 1954. Cell enlargement and cell division in excised tobacco pith tissue. Physiol. Plant. 7, 16 - 24.

JACOB, H., 1974. Untersuchungen zur Thermotherapy von Steinobstvirosen. Acta Phytomed. Heft 2. Berlin: Paul Parey Verlag.

JACOBS, G., 1973. Further tissue culture studies on the genus Rosa with special reference to the shoot tip. Ph.D. Thesis, Univ. of Natal.

JACOBS, W.P., 1954. Acropetal auxin transport and xylem regeneration - a quantitative study. Am. Nat. 88, 327 - 337.

JACOBS, G., ALLAN, P. & BORNMAN, C.H., 1969. Tissue culture studies on rose: use of shoot tip explants. I. Auxin:cytokinin effects. Agroplantae 1, 179 - 188.

JACOBS, G., ALLAN, P. & BORNMAN, C.H., 1970a. Tissue culture studies on rose: use of shoot tip explants. II. Cytokinin: gibberellin effects. Agroplantae 2, 25 - 28.

JACOBS, G., ALLAN, P. & BORNMAN, C.H., 1970b. Tissue culture studies on rose: use of shoot tip explants. III. Auxin: gibberellin effects. Agroplantae 2, 45 - 50.

JACOBS, W.P. & MORROW, I.B., 1957. A quantitative study of xylem development in the vegetative shoot apex of Coleus. Am. J. Bot. 44, 823 - 842.

JACOBS, W.P. & MORROW, I.B., 1958. Quantitative relations between stages of leaf development and differentiation of sieve tubes. Science 128, 1084 - 1085.

JáKó, N. & NITSCH, C., 1980. Wechselnder Hormonbedarf von Gewebekulturen aus Triebspitzenmeristem bei Vitis vinifera L. cv. Sultana in Abhängigkeit von der Entwicklungsphase. Mitt. Klosterneuburg 30, 231 - 237.

JAMES, D.J. & NEWTON, B., 1977. Auxin:cytokinin interactions in the in vitro micropropagation of strawberry plants. Acta Hort. 78, 321 - 331.

JELASKA, S. & SUTINA, R., 1977. Maintained culture of multiple plantlets from carnation shoot tips. Acta Hort. 78, 333 - 340.

JOHANSEN, D.A., 1940. Plant Microtechnique. New York: McGraw-Hill, Inc.

JONES, O.P., 1967. Effect of benzyl adenine on isolated apple shoots. Nature 215, 1514 - 1515.

JONES, O.P. & VINE, S.J., 1968. The culture of gooseberry shoot tips for eliminating virus. J. Hort. Sci. 43, 289 - 292.

JONES, O.P., HOPGOOD, M.E. & O'FARRELL, D., 1977. Propagation in vitro of M.26 apple rootstocks. J. Hort. Sci. 52, 235 - 238.

KARTHA, K.K., GAMBORG, O.L., CONSTABEL, F. & SHYLUK, J.P., 1974. Regeneration of cassava plants from apical meristems. Plant Sci. Letters 2, 107 - 113.

KASSANIS, B., 1957. The use of tissue culture to produce virus-free clones from infected potato varieties. Ann. Appl. Biol. 45, 422 - 427.

KASSANIS, B., 1967. Plant tissue culture. In: Methods in Virology, Vol. 1, pp. 537 - 566. Eds. K. Maramorosch and H. Koprowski. New York - London: Academic Press.

KASSANIS, B. & VARMA, A., 1967. The production of virus-free clones of some British potato varieties. Ann. Appl. Biol. 59, 447 - 450.

KIM, K.K., 1969. Meristem culture of Dendrobium (Orchidaceae). M.S. Thesis, Univ. Hawaii.

KNOP, W., 1865. Quantitative Untersuchungen über den Ernahrungsprozess der Pflanzen. Landw. Versuchs - stat. 7, 93 - 109.

KRIEDEMANN, P.E. & SMART, R.E., 1971. Effects of irradiance, temperature and leaf water potential on photosynthesis of vine leaves. Photosynthetica (Prague) 5, 6 - 15.

KRIEDEMANN, P.E., SWARD, R.J. & DOWNTON, W.J.S., 1976. Vine response to carbon dioxide enrichment during heat therapy. Aust. J. Plant Physiol. 3, 605 - 618.

KROEMER, K., 1923. Organographie, Anatomie und Physiologie der Rebe. In: Handbuch des Weinbaues und die Kellerwirtschaft, pp. 255 - 286. Eds. F. von Babo and E. Mach. Berlin: Parey.

KUMAR, S., 1963. Tuberization in vitro and physiological studies with potato callus tissue. Ph.D. Thesis, Univ. Wisconsin.

LABRUN-PEREMANS, G., 1966. Culture in vitro des meristems apicaux d'oeillet. Agriculture 1, 97 - 103.

LACKEY, C.F., 1946. Occurrence of curly-top virus in meristematic tissue. Phytopathology 36, 462 - 468.

LARSEN, E.C., 1974. Heat inactivation of viruses in apple cl MM 109 by use of daily temperature cycles. Tidsskr. Planteavl 78, 422 - 428.

LAWALRéE, A., 1948. Histogénèse florale et végétative chez quelques Composées. Cellule 52, 215 - 294.

LEOPOLD, A.C., 1964. Differential growth. In: Plant Growth and Development. New York: McGraw-Hill Book Co.

LIMASETT, P. & CORNUET, P., 1949. Recherche de virus de la mosaïque du tabac (Marmor Tabaci, Holmes) dans les méristèmes des plantes infectées. C.R. Acad. Sc. 228, 1971 - 1972.

MA, S.S. & WANG, S.O., 1977. Clonal multiplication of Azaleas through tissue culture. Acta Hort. 78, 209 - 215.

MALNASSY, P. & ELLISON, J.H., 1970. Asparagus tetraploids from callus tissue. Hort. Sci. 5, 444 - 445.

Mc GAHAN, M.W., 1955. Vascular differentiation in the vegetative shoot of Xanthium chinese. Am. J. Bot. 42, 132 - 140.

McGREW, J.R., 1965. Eradication of latent C virus in the Suwannee variety of strawberry by heat plus excised runner-tip culture. Phytopathology 55, 480 - 481.

MEER, F.A. van der, 1967. Virusziekten in appel en peer. Ann. Rep. Inst. Phytopath. Res., Wageningen 1966, 100.

MEKERS, O., 1977. In vitro propagation of some Tillandsioideae (Bromeliaceae). Acta Hort. 78, 311 - 317.

MELLOR, F.C. & FITZPATRICK, R.E., 1961. Strawberry viruses. Can. Plant Dis. Surv. 41, 218 - 255.

MELLOR, F.C. & STACE-SMITH, R., 1977. Virus-free potatoes by tissue culture. In: Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture, pp. 616 - 635. Eds. J. Reinert and Y.P.S. Bajaj. Berlin: Springer-Verlag.

MILLER, P.W. & BELKENGREN, R.O., 1963. Elimination of yellow edge, crinckle and vein-banding viruses and certain other complexes from strawberries by excision and culturing of apical meristems. Plant Dis. Rep. 47, 298 - 300.

MILLER, H.A. & WETMORE, R.H., 1946. Studies in the developmental anatomy of Phlox drummondii H. The apices of the mature plant. Am. J. Bot. 33, 1 - 10.

MOREL, G., 1960. Producing virus-free cymbidiums. Am. Orchid Soc. Bull. 29, 495 - 497.

MOREL, G., 1964a. La culture in vitro du méristéme apical. Rev. Cytol. Biol. Vég. 27, 307 - 314.

MOREL, G., 1964b. Régénération des variétés virosées par la culture des méristème apicaux. Rev. Horticole 733 - 740.

MOREL, G., 1964c. Tissue culture - a new means of clonal propagation of orchids. Am. Orchid Soc. Bull. 31, 473 - 478.

MOREL, G. & MARTIN, C., 1952. Guérison de dahlias atteints d'une maladie à virus. C.R. Acad. Sci. Paris 235, 1324 - 1325.

MOREL, G. & MARTIN, C., 1955. Guérison de pommes de terre atteintics de maladies a virus. C.R. hébd. Séance. Acad. Agric. Fr. 41, 472 - 474.

MORI, K. & HOSOKAWA, D., 1977. Localization of viruses in apical meristem and production of virus-free plants by means of meristem and tissue culture. Acta Hort. 78, 389 - 396.

MULLIN, R.H., FRAZIER, N.W. & SCHLEGEL, D.E., 1975. Meristem culture of Fragaria chiloensis infected with strawberry pallidosis. Plant Dis. Rep. 59, 268.

MULLIN, R.H., SMITH, S.H., FRAZIER, N.W., SCHLEGEL, D.E. & McCALL, S.R., 1974. Meristem culture frees strawberries of mild yellow edge, pallidosis and mottle diseases. Phytopathology 64, 1425 - 1429.

MURASHIGE, T., 1966. Principles of in vitro culture. Proc. Inter. Plant Prop. Soc. 16, 80 - 87.

MURASHIGE, T., 1974. Plant propagation through tissue cultures. Ann. Rev. Plant Physiol. 25, 135 - 166.

MURASHIGE, T., 1977. Plant cell and organ cultures as horticultural practices. Acta Hort. 78, 17 - 30.

MURASHIGE, T. & SKOOG., F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15, 473 - 497.

NISHIZAWA, T. & NISHI, Y., 1966. Studies on the production of virus-free lily plants by means of meristem culture. Bull. Kyushu Agr. Exp. Sta. 12, 139 - 157.

NYLAND, G., 1960. Heat inactivation of stone fruit ringspot virus. Phytopathology 50, 380 - 382.

NYLAND, G. & GOHEEN, A.C., 1969. Heat therapy of virus diseases of perennial plants. Ann. Rev. Phytopath. 7, 331 - 354.

ORFFER, C.J., 1956. Ontogenetic and experimental studies on the establishment of the graft union in the grapevine. Ph.D. Thesis, Univ. California.

OS, H. van, 1964. Production of virus-free carnations by means of meristem culture. Neth. J. Plant Path. 70, 18 - 26.

PENNAZIO, S., 1975. Effects of adenine and kinetin on development of carnation meristem tips cultured in vitro. J. Hort. Sci. 50, 161 - 164.

PHILIPSON, W.R., 1949. The ontogeny of the shoot apex in dicotyledons. Biol. Rev. 24, 21 - 50.

PHILLIPS, D.J., 1968. Carnation shoot tip culture. Colorado State Univ. Exp. Sta. Tech. Bull. 102.

PHILLIPS, D.J. & MATTHEWS, G.J., 1964. Growth and development of carnation shoot tips *in vitro*. Bot. Gaz. 125, 7 - 12.

PILLAI, S.K. & HILDEBRANDT, A.C., 1968. Geranium plants differentiated *in vitro* from stem tip and callus cultures. Plant Dis. Rep. 52, 600 - 601.

PILLAI, S.K. & HILDEBRANDT, A.C., 1969. Induced differentiation of geranium plants from undifferentiated callus *in vitro*. Am. J. Bot. 56, 52 - 58.

POOL, R.M., 1974. Relation of cytokinins, abscisic acid and metabolites of abscisic acid to vine growth and leaf function in *Vitis*. Ph.D. Thesis, Univ. Cornell.

POOL, R.M., 1975. Effect of cytokinin on *in vitro* development of Concord flowers. Am. J. Enol. Vitic. 26, 43 - 46.

POPHAM, R.A., 1951. Principal types of vegetative shoot apex organization in vascular plants. Ohio Jour. Sci. 51, 249 - 270.

POPHAM, R.A. & CHAN, A.P., 1950. Zonation in the vegetative stem tip of *Chrysanthenum morifolium* Bailey. Am. J. Bot. 37, 476 - 484.

POSNETTE, A.F. & CROPLEY, R., 1956. Apple mosaic viruses. Host reactions and strain interference. J. Hort. Sci. 31, 119 - 133.

POSNETTE, A.F. & CROPLEY, R., 1958. Heat treatment for the inactivation of strawberry viruses. J. Hort. Sci. 33, 282 - 288.

PRATT, C., 1959. Radiation damage in shoot apices of Concord grape. Am. J. Bot. 46, 102 - 109.

PRATT, C., 1974. Vegetative anatomy of cultivated grapes - A review. Am. J. Enol. Vitic. 25, 131 - 150.

PREIL, W. & ENGELHARDT, M., 1977. Meristem culture of Azaleas (Rhododendron Simsii). Acta Hort. 78, 203 - 208.

QUAK, F., 1957. Meristeemcultuur, gecombineerd met warmtebehandeling voor het verkrijgen van virusvrije anjers. Tidsskr. Pl. - ziekten 63, 13 - 14.

QUAK, F., 1977. Meristem culture and virus-free plants. In: Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture, pp. 598 - 615. Eds. J. Reinert and Y.P.S. Bajaj. Berlin: Springer-Verlag.

QUOIRIN, M. & LEPOIVRE, P., 1977. Etude de milieux adaptés aux cultures in vitro de Prunus. Acta Hort. 78, 437 - 442.

REEVE, R.M., 1948. The "tunica-corpus" concept and development of shoot apices in certain dycotyledons. Am. J. Bot. 35, 65 - 75.

REYNOLDS, S.L. & CORBETT, M.K., 1980. Electron microscopy of viral-like particles in Chardonnay grapevines. Proc. 7th meeting ICVG, Niagara Falls, Canada, pp. 205 - 210.

RIVES, M., 1970. Complete cure of a whole plant of Vitis rupestris cv. du Lot (St. Georges) of grapevine fanleaf by thermotherapy. Plant Dis. Rep. 54, 915 - 916.

ROBERTS, D.A., CHRISTIE, R.G. & ARCHER, M.C., 1970. Infection of apical initials in tobacco shoot meristems by tobacco ringspot virus. Virology 42, 217 - 220.

ROMBERG, J.A., 1963. Meristems, growth and development in woody plants. U.S. Dept. Agric. For. Serv. Tech. Bull. 1293.

ROUFFA, A.S. & GUNCKEL, J.E., 1951. A comparative study of vegetative shoot apices in the Rosaceae. Am. J. Bot. 38, 290 - 300.

SAGAWA, Y., 1976. Potential of *in vitro* culture techniques for improvement of floricultural crops. Acta Hort. 63, 61 - 66.

SCHMIDT, A., 1924. Histologische Studien an phanerogamen Vegetationspunkten. Bot. Arch. 8, 345 - 404.

SEELIGER, I., 1954. Studien am Sprossvegetationskegel von *Thujopsis dolabrata* (L.f.) Sieb. et Zucc. Flora 142, 183 - 212.

SHABDE, M. & MURASHIGE, T., 1977. Hormonal requirements of excised *Dianthus caryophyllus* L. shoot apical meristems *in vitro*. Am. J. Bot. 64, 443 - 448.

SHEFFIELD, F.M., 1942. Presence of virus in the primordial meristem. Ann. Appl. Biol. 29, 16 - 17.

SIMONSEN, J. & HILDEBRANDT, A.C., 1971. *In vitro* growth and differentiation of *Gladiolus* plants from callus cultures. Can. J. Bot. 49, 1817 - 1819.

SIMONSEN, J. & HILDEBRANDT, A.C., 1972. Anther and stem tissue callus from healthy and potato virus X infected potatoes. Tidsskr. Planteavl. 76, 127 - 131.

SINGHA, S., 1980. In vitro propagation of 'Seckel' Pear. Proceedings of the Conference on Nursery Production of Fruit Plants through Tissue Culture - Applications and Feasibility, Beltsville, Maryland, pp. 59 - 63.

SKOOG, F. & TSUI, C., 1948. Chemical control of growth and bud formation in tobacco stem segments and callus cultured in vitro. Am. J. Bot. 35, 782 - 787.

SKOOG, F. & MILLER, C.O., 1957. Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symp. Soc. Exp. Biol. 11, 118 - 130.

SMITH, R.H. & MURASHIGE, T., 1970. In vitro development of the isolated shoot apical meristem of angiosperms. Am. J. Bot. 57, 562 - 568.

SNEDECOR, G.W. & COCHRAN, W.G., 1967. Statistical Methods, 6th Ed. Ames: Iowa State Univ. Press.

STACE-SMITH, R. & MELLOR, F.C., 1968a. Eradication of potato viruses X and S by thermotherapy and axillary bud culture. Phytopathology 58, 199 - 203.

STACE-SMITH, R. & MELLOR, F.C., 1968b. Virus-free potatoes: a reality. Can. Agric. 13, 2 - 6.

STELLMACH, G., 1980a. Propagation of healthy plants from green shoot tips produced on vines infected with Nepo-viruses. Pflkrankh. PflSchutz 87, 92 - 96.

STELLMACH, G., 1980b. Moderate heat propagation of grapevines for eliminating graft transmissible disorders. Proc. 7th meeting ICVG, Niagara Falls, Canada, pp. 325 - 328.

STERLING, C., 1947. Organization of the shoot of Pseudotsuga taxifolia (Lamb.) Britt. II. Vascularization. Am. J. Bot. 34, 272 - 280.

STONE, O.M., 1963. Factors affecting the growth of carnation plants from shoot apices. Ann. Appl. Biol. 52, 199 - 209.

STONE, O.M., 1968. The elimination of four viruses from carnation and sweet william by meristem-tip culture. Ann. Appl. Biol. 119 - 122.

SUNDERLAND, N., 1961. Cell division and expansion in the growth of the shoot apex. J. Exp. Bot. 12, 446 - 457.

SUSSEX, I.M., 1955. Morphogenesis in Solanum tuberosum L.: apical structure and developmental pattern of the juvenile shoot. Phytomorphology 5, 253 - 273.

SVOBODOVA, J., 1966. Elimination of viruses by means of callus tissue cultures. In: Viruses in Plants, pp. 48 - 53. Eds. A.B.R. Beenstra and J. Dykstra. New York - London: John Wiley & Sons.

TABACHNIK, L. & KESTER, D.E., 1977. Shoot culture for almond and almond-peach hybrid clones in vitro. Hort. Sci. 12, 545 - 547.

THEILER, R., 1977. In vitro culture of shoot tips of Pelargonium species. Acta Hort. 78, 403 - 414.

THOMPSON, M.M. & OLMO, H.P., 1963. Cytohistological studies of cytochimeric and tetraploid grapes. Am. J. Bot. 50, 901 - 906.

THOMSON, A.D., 1956. Studies on the effect of malachite green on potato virus X and Y. Aust. J. Agric. Res. 7, 428 - 434.

TUCKER, S.C. & HOEFERT, L.L., 1968. Ontogeny of the tendril in Vitis vinifera. Am. J. Bot. 55, 1110 - 1119.

VAUGHAN, J.G., 1952. Structure of the angiosperm apex. Nature 169, 458 - 459.

VIALA, P. & PéCHOUTRE, P., 1910. Morphologie du genre Vitis. In: Traité général de Viticulture ampélographie, Vol. 1, pp. 198 - 298. Eds. P. Viala and V. Vermorel. Paris: Masson.

VINE, S.J., 1968. Improved culture of apical tissues for production of virus-free strawberries. J. Hort. Sci. 43, 293 - 297.

VINE, S.J. & JONES, O.P., 1969. The culture of shoot tips of hop (Humulus lupulus) to eliminate viruses. J. Hort. Sci. 44, 281 - 284.

VOLLER, A., BIDWELL, D.E. & BARTLETT, A., 1977. The enzyme-linked immunosorbent assay. Guernsey, U.K.: Flowline Press.

VOLLMER, W., 1976. Der Cytokiningehalt in den Vegetativen Organen der Rebe. Ph.D. Dissertation, Univ. Hohenheim.

WALKEY, D.G.A., 1968. The production of virus-free rhubarb by apical tip culture. J. Hort. Sci. 43, 283 - 287.

WALKEY, D.G.A., 1978. In vitro methods for virus elimination. In: Frontiers of Plant Tissue Culture, pp. 245 - 254. Ed. T.A. Thorpe. Calgary:Inter. Assoc. Plant Tissue Culture.

WALKEY, D.G.A., 1980. Production of virus-free plants by tissue culture. In: Tissue Culture Methods for Plant Pathologists, pp. 109 - 117. Eds. D.S. Ingram and J.P. Helgeson. Oxford: Blackwell Scientific Publications.

WALKEY, D.G.A. & COOPER, V.C., 1975. Effect of temperature on virus eradication and growth of infected tissue cultures. Ann. Appl. Biol. 80, 185 - 190.

WALKEY, D.G.A. & FREEMAN, G.H., 1977. Inactivation of cucumber mosaic virus in cultured tissues of Nicotiana rustica L. by diurnal alternating periods of high and low temperature. Ann. Appl. Biol. 87, 375 - 382.

WALKEY, D.G.A. & WEBB, M.J.W., 1968. Viruses in plant apical meristems. J. Gen. Virol. 3, 311 - 313.

WALKEY, D.G.A. & WEBB, M.J.W., 1970. Tubular inclusion bodies in plants infected with viruses of the Nepo-type. J. Gen. Virol. 7, 159 - 166.

WALKEY, D.G.A., FITZPATRICK, J. & WOOLFITT, J.M.G., 1969. The inactivation of virus in cultured shoot tips of Nicotiana rustica L. J. Gen. Virol. 5, 237 - 241.

WANG, P.J. & HUANG, L.C., 1975. Callus cultures from potato tissues and the exclusion of potato virus X from plants regenerated from stem tips. Can. J. Bot. 53, 2565 - 2567.

WANG, P.J. & HU, C.Y., 1980. Regeneration of virus-free plants through in vitro culture. Advances in Biochemical Engineering 18, Plant Cell Cultures II, 61 - 99.

WETMORE, R.H., 1959. Morphogenesis in plants: A new approach. Am. Sci. 47, 326 - 340.

WETMORE, R.H. & RIER, J.P., 1963. Experimental induction of vascular tissues in callus of angiosperms. Am. J. Bot. 50, 418 - 430.

WETMORE, R.H. & SOROKIN, S., 1955. On the differentiation of xylem. J. Arnold Arboetum 36, 305 - 317.

WHITE, P.R., 1934. Multiplication of the viruses of tobacco and ancuba mosaic in growing excised tomato roots. Phytopathology 24, 1003 - 1011.

WHITE, P.R., 1943. A Handbook of Plant Tissue Culture. Lancaster: Jaques Cattell Press.

WHITE, P.R., 1954. The Cultivation of Animal and Plant Cells, 1st Ed. New York: Ronald Press Co.

WHITE, P.R., 1963. The Cultivation of Animal and Plant Cells, 2nd Ed. New York: Ronald Press Co.

WICKSON, M.E. & THIMANN, K.V., 1958. The antagonism of auxin and kinetin in apical dominance. Physiol. Plant. 11, 62 - 74.

WILMAR, J.C., HILDEBRANDT, A.C. & RIKER, A.J., 1964. Iron nutrition for growth and chlorophyll development of some plant tissue cultures. Nature 202, 1235.

WINTON, L.L., 1968. The rooting of liquid-grown Aspen callus. Am. J. Bot. 55, 159 - 167.

WINTON, L.L., 1970. Shoot and tree production from aspen tissue cultures. Am. J. Bot. 57, 904 - 909.

WOLFSWINKEL, L.D., 1965. Tissue culture as a possible means to healthier grape propagation material. Proc. Int. Conf. Virus Vector Perennial Hosts, Davis, California, pp. 318 - 322.

WOLTER, K.E., 1968. Root and shoot initiation in Aspen callus cultures. Nature 219, 509 - 510.

YIE, S. & LIAW, I., 1977. Plant regeneration from shoot tips and callus of papaya. In vitro 13, 564 - 568.

ZIMMERMAN, R.H. & BROOME, O.C., 1980. Blueberry micropropagation. Proceedings of the Conference on Nursery Production of Fruit Plants through Tissue Culture - Applications and Feasibility, Beltsville, Maryland, pp. 44 - 47.

APPENDIX

APPENDIX 1.1 - The effect of different concentration ratios of kinetin and indole-3-acetic acid (IAA) on shoot elongation, total shoot mass, leaf expansion and percentages of explants with expanded leaves derived from shoot apices of Chenin blanc cultured in vitro

Treatment (mg/ℓ) KIN	IAA	Elongation (mm)	Shoot mass (mg)	Number of expanded leaves	% explants with expanded leaves
0,0	0,0	3,29 n	9,83 k	0,00 g	0 f
0,0	0,5	3,76 mn	11,23 jk	0,00 g	0 f
0,0	1,0	4,29 lm	11,41 jk	0,00 g	0 f
0,0	2,0	4,96 jkl	12,32 hijk	0,00 g	0 f
0,0	4,0	5,00 ijkl	16,94 hijk	0,00 g	0 f
0,0	8,0	5,02 ijkl	17,25 hijk	0,00 g	0 f
0,0	16,0	4,67 kl	11,61 ijk	0,00 g	0 f
0,25	0,0	5,01 ijkl	12,62 hijk	0,00 g	0 f
0,25	0,5	5,03 ijkl	12,87 hijk	0,20 g	20 ef
0,25	1,0	5,59 g-k	15,59 hijk	0,25 g	25 def
0,25	2,0	5,79 c-j	16,95 hijk	0,25 g	25 def
0,25	4,0	5,85 c-j	16,97 hijk	0,25 g	25 def
0,25	8,0	5,92 b-j	17,29 hijk	0,25 g	25 def
0,25	16,0	5,13 h-l	13,05 hijk	0,00 g	0 f
0,5	0,0	5,34 hijk	16,59 hijk	0,00 g	0 f
0,5	0,5	5,62 f-k	17,62 hijk	0,40 fg	40 cde
0,5	1,0	5,76 d-j	17,77 hijk	0,40 fg	40 cde
0,5	2,0	5,80 c-j	17,90 hijk	0,40 fg	40 cde
0,5	4,0	5,90 b-j	18,32 hijk	0,40 fg	40 cde
0,5	8,0	5,93 b-j	18,56 hijk	0,40 fg	40 cde
0,5	16,0	5,47 hijk	16,67 hijk	0,15 g	15 ef
1,0	0,0	5,62 f-k	18,39 hijk	1,00 cdef	55 cd
1,0	0,5	5,72 d-j	18,50 hijk	1,00 cdef	55 cd
1,0	1,0	5,79 c-j	23,15 hijk	1,00 cdef	60 c
1,0	2,0	5,96 b-j	24,17 ghij	1,00 cdef	60 c
1,0	4,0	6,04 a-j	25,47 fghi	1,00 cdef	60 c
1,0	8,0	6,13 a-i	25,75 fgh	1,00 cdef	65 bc
1,0	16,0	5,67 e-k	17,33 hijk	0,85 ef	45 cde
2,0	0,0	5,75 d-j	33,90 efg	1,25 cde	90 ab
2,0	0,5	5,96 b-j	34,37 efg	1,25 cde	90 ab
2,0	1,0	6,17 a-h	34,97 ef	1,25 cde	100 a
2,0	2,0	6,18 a-h	39,43 cde	1,50 bcd	100 a
2,0	4,0	6,20 a-h	40,43 cde	1,50 bcd	100 a
2,0	8,0	6,23 a-h	40,46 cde	1,55 bc	100 a
2,0	16,0	5,95 b-j	36,59 de	0,85 ef	80 ab
4,0	0,0	6,68 a-g	44,09 bcde	1,85 ab	100 a
4,0	0,5	6,70 a-g	44,75 bcde	1,95 ab	100 a
4,0	1,0	6,70 a-g	44,84 bcde	2,00 ab	100 a
4,0	2,0	6,74 a-f	47,52 bcd	2,05 ab	100 a
4,0	4,0	6,77 a-f	47,87 bcd	2,15 a	100 a
4,0	8,0	6,80 a-e	48,38 bcd	2,15 a	100 a
4,0	16,0	5,98 b-j	43,84 bcde	0,90 def	75 ab
8,0	0,0	6,84 abcd	45,34 bcde	2,05 ab	100 a
8,0	0,5	6,85 abcd	49,59 bc	2,15 a	100 a
8,0	1,0	6,91 abc	53,64 b	2,25 a	100 a
8,0	2,0	6,93 abc	70,64 a	2,25 a	100 a
8,0	4,0	7,07 a	71,60 a	2,30 a	100 a
8,0	8,0	6,99 ab	71,28 a	2,30 a	100 a
8,0	16,0	6,03 a-j	63,25 a	1,35 cde	70 abc

a-n: Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level.

APPENDIX 1.2 - The effect of different concentrations of kinetin and indole-3-acetic acid (IAA) on shoot elongation, total shoot mass, leaf expansion and percentages of explants with expanded leaves derived from shoot apices of US 1-9 cultured in vitro

Treatment (mg/ℓ) KIN : IAA		Elongation (mm)	Shoot mass (mg)	Number of expanded leaves	% explants with expanded leaves
0,0	0,0	3,32 f	10,19 i	0,00 j	0 e
0,0	0,5	3,35 f	11,76 i	0,00 j	0 e
0,0	1,0	3,36 f	11,77 i	0,00 j	0 e
0,0	2,0	3,39 f	11,89 i	0,00 j	0 e
0,0	4,0	3,40 f	12,06 hi	0,00 j	0 e
0,0	8,0	3,38 f	11,92 i	0,00 j	0 e
0,0	16,0	3,30 f	11,45 i	0,00 j	0 e
0,25	0,0	4,42 de	17,47 ghi	0,00 j	0 e
0,25	0,5	4,43 cde	17,84 ghi	0,00 j	0 e
0,25	1,0	4,44 cde	17,95 ghi	0,00 j	0 e
0,25	2,0	4,54 cde	17,98 ghi	0,00 j	0 e
0,25	4,0	4,54 cde	17,98 ghi	0,00 j	0 e
0,25	8,0	4,42 de	17,65 ghi	0,00 j	0 e
0,25	16,0	3,34 f	15,09 hi	0,00 j	0 e
0,5	0,0	4,65 cde	20,38 fghi	0,50 ij	50 c
0,5	0,5	4,66 cde	20,94 e-i	0,65 i	55 bc
0,5	1,0	4,67 cde	21,29 d-i	0,70 i	55 bc
0,5	2,0	4,69 cde	21,32 d-i	0,70 i	55 bc
0,5	4,0	4,69 cde	21,45 d-i	0,70 i	70 bc
0,5	8,0	4,47 cde	19,98 fghi	0,45 ij	25 de
0,5	16,0	3,35 f	15,33 hi	0,0 j	0 e
1,0	0,0	4,90 cde	29,35 d-i	1,25 h	75 abc
1,0	0,5	4,92 cde	29,45 d-i	1,25 h	75 abc
1,0	1,0	4,93 cde	29,55 d-i	1,30 h	75 abc
1,0	2,0	4,94 cde	30,36 d-i	1,35 h	75 abc
1,0	4,0	4,96 cde	30,44 d-i	1,50 h	75 abc
1,0	8,0	4,80 cde	29,13 d-i	0,45 ij	30 d
1,0	16,0	4,15 e	17,30 ghi	0,00 j	0 e
2,0	0,0	5,23 cd	42,13 d-h	1,95 g	95 a
2,0	0,5	5,25 cd	42,44 defg	1,95 g	95 a
2,0	1,0	5,30 cd	43,83 defg	2,00 g	100 a
2,0	2,0	5,31 cd	45,70 def	2,05 g	100 a
2,0	4,0	5,33 c	46,92 d	2,10 fg	100 a
2,0	8,0	5,23 cd	45,36 def	1,15 h	50 c
2,0	16,0	4,21 e	20,21 fghi	0,05 j	5 e
4,0	0,0	7,08 b	85,00 c	2,45 def	100 a
4,0	0,5	7,09 b	87,13 c	2,50 de	100 a
4,0	1,0	7,10 b	88,27 c	2,65 cd	100 a
4,0	2,0	7,12 b	90,16 c	2,95 bc	100 a
4,0	4,0	7,13 b	91,68 c	3,05 ab	100 a
4,0	8,0	6,97 b	86,88 c	2,20 efg	80 ab
4,0	16,0	4,30 e	29,46 d-i	0,15 j	15 de
8,0	0,0	8,82 a	261,22 b	3,35 ab	100 a
8,0	0,5	8,84 a	265,07 b	3,35 ab	100 a
8,0	1,0	8,86 a	274,53 b	3,35 ab	100 a
8,0	2,0	8,88 a	280,05 b	3,45 a	100 a
8,0	4,0	8,90 a	296,37 a	3,45 a	100 a
8,0	8,0	8,44 a	278,99 b	2,65 cd	90 a
8,0	16,0	4,72 cde	46,85 de	0,25 ij	20 de

a-j: Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level.

APPENDIX 2.1 - The effect of different concentrations of kinetin and 1-naphthaleneacetic acid (NAA) on shoot elongation, total shoot mass, callus mass, leaf expansion and percentages of explants with expanded leaves derived from shoot apices of Chenin blanc cultured in vitro

Treatment (mg/ℓ) KIN	NAA	Elongation (mm)	Shoot mass (mg)	Callus mass (mg)	Number of expanded leaves	% explants with expanded leaves
0,0	0,0	3,38 efg	10,80 f	0,00 c	0,00 e	0 h
0,0	0,1	3,27 e-i	9,93 f	0,00 c	0,00 e	0 h
0,0	0,5	3,06 e-i	8,20 f	0,00 c	0,00 e	0 h
0,0	1,0	3,01 e-i	7,80 f	8,31 abc	0,00 e	0 h
0,0	2,0	2,92 fghi	7,78 f	12,70 ab	0,00 e	0 h
0,0	4,0	2,87 fghi	7,55 f	13,00 ab	0,00 e	0 h
0,0	8,0	2,43 i	6,00 f	11,00 ab	0,00 e	0 h
0,25	0,0	4,70 d	11,25 f	0,00 c	0,10 e	10 g
0,25	0,1	3,28 efgh	10,35 f	0,00 c	0,00 e	0 h
0,25	0,5	3,22 e-i	8,30 f	0,00 c	0,00 e	0 h
0,25	1,0	3,01 e-i	7,95 f	8,50 abc	0,0 e	0 h
0,25	2,0	2,98 e-i	7,91 f	13,55 a	0,00 e	0 h
0,25	4,0	2,96 e-i	7,61 f	14,00 a	0,00 e	0 h
0,25	8,0	2,44 hi	6,20 f	9,50 abc	0,00 e	0 h
0,5	0,0	5,14 c	17,77 ef	0,00 c	0,15 e	15 f
0,5	0,1	3,30 efg	11,15 f	0,00 c	0,00 e	0 h
0,5	0,5	3,25 e-i	9,46 f	0,00 c	0,00 e	0 h
0,5	1,0	3,10 e-i	8,05 f	8,73 abc	0,00 e	0 h
0,5	2,0	3,00 e-i	7,95 f	12,65 ab	0,00 e	0 h
0,5	4,0	2,98 e-i	7,68 f	13,50 a	0,00 c	0 h
0,5	8,0	2,68 ghi	6,30 f	8,00 abc	0,00 e	0 h
1,0	0,0	5,57 b	30,45 c	0,00 c	1,00 d	70 c
1,0	0,1	3,47 efg	11,82 f	0,00 c	0,00 e	0 h
1,0	0,5	3,32 efg	9,58 f	6,80 abc	0,00 e	0 h
1,0	1,0	3,20 e-i	8,22 f	9,19 abc	0,00 e	0 h
1,0	2,0	3,10 e-i	8,00 f	11,73 ab	0,00 e	0 h
1,0	4,0	3,00 e-i	7,80 f	8,00 abc	0,00 e	0 h
1,0	8,0	2,69 ghi	6,50 f	7,50 abc	0,00 e	0 h
2,0	0,0	5,79 ab	37,46 b	0,00 c	1,40 c	85 b
2,0	0,1	3,79 e	13,09 f	4,95 abc	0,00 e	0 h
2,0	0,5	3,40 efg	9,61 f	11,06 ab	0,00 e	0 h
2,0	1,0	3,25 e-i	8,35 f	11,96 ab	0,00 e	0 h
2,0	2,0	3,15 e-i	8,10 f	11,50 ab	0,00 e	0 h
2,0	4,0	3,05 e-i	7,95 f	7,00 abc	0,00 e	0 h
2,0	8,0	2,79 fghi	6,95 f	6,90 abc	0,00 e	0 h
4,0	0,0	6,12 a	46,03 a	0,00 c	2,10 b	100 a
4,0	0,1	4,34 d	22,56 de	5,00 abc	0,25 e	25 e
4,0	0,5	3,53 efg	9,81 f	5,65 abc	0,00 e	0 h
4,0	1,0	3,30 efg	8,88 f	7,78 abc	0,00 e	0 h
4,0	2,0	3,20 e-i	8,50 f	8,46 abc	0,00 e	o h
4,0	4,0	3,10 e-i	8,25 f	6,00 abc	0,00 e	0 h
4,0	8,0	2,85 fghi	7,10 f	5,00 abc	0,00 e	0 h
8,0	0,0	6,20 a	51,46 a	0,00 c	2,30 a	100 a
8,0	0,1	4,50 d	25,24 cd	0,00 c	0,45 e	45 d
8,0	0,5	3,63 ef	9,99 f	3,80 bc	0,00 e	0 h
8,0	1,0	3,48 efg	9,46 f	7,11 abc	0,00 e	0 h
8,0	2,0	3,24 e-i	8,96 f	7,98 abc	0,00 e	0 h
8,0	4,0	3,18 e-i	8,70 f	4,00 bc	0,00 e	0 h
8,0	8,0	2,90 fghi	7,15 f	3,80 bc	0,00 e	0 h

a-i: Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level.

APPENDIX 2.2 - The effect of kinetin and 1-naphthaleneacetic acid (NAA) on shoot elongation, total shoot mass, callus mass, leaf expansion and percentages of explants with expanded leaves derived from shoot apices of US 1-9 cultured in vitro

Treatment (mg/ℓ) KIN	NAA	Elongation (mm)	Shoot mass (mg)	Callus mass (mg)	Number of expanded leaves	% explants with expanded leaves
0,0	0,0	3,40 ijk	10,04 g	0,00 c	0,00 g	0 d
0,0	0,1	3,04 j-n	8,51 g	0,00 c	0,00 g	0 d
0,0	0,5	3,00 j-n	7,89 g	0,00 c	0,00 g	0 d
0,0	1,0	2,90 j-n	7,14 g	4,98 c	0,00 g	0 d
0,0	2,0	2,63 klmn	6,71 g	15,00 c	0,00 g	0 d
0,0	4,0	2,52 mn	6,59 g	35,00 c	0,00 g	0 d
0,0	8,0	2,46 n	6,54 g	53,39 c	0,00 g	0 d
0,25	0,0	4,44 efg	13,05 fg	0,00 c	0,00 g	0 d
0,25	0,1	3,40 ijk	8,81 g	0,00 c	0,00 g	0 d
0,25	0,5	3,29 i-m	8,24 g	0,00 c	0,00 g	0 d
0,25	1,0	2,94 j-n	7,89 g	8,30 c	0,00 g	0 d
0,25	2,0	2,84 j-n	7,30 g	46,06 c	0,00 g	0 d
0,25	4,0	2,70 klmn	6,88 g	59,59 c	0,00 g	0 d
0,25	8,0	2,60 lmn	6,84 g	176,72 c	0,00 g	0 d
0,5	0,0	4,88 de	21,63 efg	0,00 c	0,35 f	35 c
0,5	0,1	3,73 hi	9,62 g	0,00 c	0,00 g	0 d
0,5	0,5	3,60 hij	8,80 g	0,00 c	0,00 g	0 d
0,5	1,0	3,33 ijkl	8,15 g	20,10 c	0,00 g	0 d
0,5	2,0	2,92 j-n	7,76 g	170,05 c	0,00 g	0 d
0,5	4,0	2,71 klmn	7,05 g	178,30 c	0,00 g	0 d
0,5	8,0	2,60 lmn	6,94 g	227,96 c	0,00 g	0 d
1,0	0,0	4,99 d	29,75 ef	0,00 c	1,20 d	80 b
1,0	0,1	4,21 fgh	15,52 efg	0,00 c	0,00 g	0 d
1,0	0,5	3,80 ghi	12,80 fg	1 709,00 b	0,00 g	0 d
1,0	1,0	3,70 hi	9,40 g	1 886,31 ab	0,00 g	0 d
1,0	2,0	2,93 j-n	8,50 g	1 746,50 b	0,00 g	0 d
1,0	4,0	2,92 j-n	8,10 g	186,22 c	0,00 g	0 d
1,0	8,0	2,81 klmn	7,50 g	233,78 c	0,00 g	0 d
2,0	0,0	5,60 c	43,80 d	0,00 c	1,90 c	95 a
2,0	0,1	4,46 ef	18,05 efg	22,85 c	0,00 g	0 d
2,0	0,5	3,95 fghi	18,00 efg	1 747,91 b	0,00 g	0 d
2,0	1,0	3,76 hi	17,50 efg	2 457,35 a	0,00 g	0 d
2,0	2,0	2,94 j-n	14,70 efg	1 920,63 ab	0,00 g	0 d
2,0	4,0	2,92 j-n	10,20 g	1 925,40 ab	0,00 g	0 d
2,0	8,0	2,90 j-n	8,60 g	243,76 c	0,00 g	0 d
4,0	0,0	6,96 b	81,50 c	0,00 c	2,40 b	100 a
4,0	0,1	5,15 d	31,47 e	19,55 c	0,00 g	0 d
4,0	0,5	3,98 fghi	29,34 ef	68,30 c	0,00 g	0 d
4,0	1,0	3,87 fghi	22,68 efg	225,80 c	0,00 g	0 d
4,0	2,0	2,95 j-n	20,38 efg	324,22 c	0,00 g	0 d
4,0	4,0	2,94 j-n	14,49 efg	356,39 c	0,00 g	0 d
4,0	8,0	2,92 j-n	12,25 fg	64,69 c	0,00 g	0 d
8,0	0,0	8,64 a	251,45 a	0,00 c	3,40 a	100 a
8,0	0,1	6,60 b	147,84 b	0,00 c	0,95 e	75 b
8,0	0,5	4,18 fgh	52,05 d	0,00 c	0,25 fg	10 d
8,0	1,0	3,95 fghi	28,72 ef	8,86 c	0,00 g	0 d
8,0	2,0	3,00 j-n	26,48 efg	25,88 c	0,00 g	0 d
8,0	4,0	2,94 j-n	19,40 efg	8,58 c	0,00 g	0 d
8,0	8,0	2,94 j-n	18,24 efg	8,10 c	0,00 g	0 d

a-n: Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level.

APPENDIX 3.1 - The effect of different concentration ratios of kinetin and indole-3-butyric acid (IBA) on shoot elongation, total shoot mass, callus mass leaf expansion and percentages of explants with expanded leaves derived from shoot apices of Chenin blanc cultured in vitro

Treatment (mg/ℓ) KIN	: IBA	Elongation (mm)	Shoot mass (mg)	Callus mass (mg)	Number of expanded leaves	% explants with expanded leaves
0,0	0,0	3,36 q-u	10,20 l-q	0,00 e	0,00 g	0 h
0,0	0,1	3,28 q-u	9,96 m-q	0,00 e	0,00 g	0 h
0,0	0,5	3,10 stu	9,85 m-q	0,00 e	0,00 g	0 h
0,0	1,0	3,05 stu	9,55 opq	0,00 e	0,00 g	0 h
0,0	2,0	3,00 tu	8,94 opq	0,00 e	0,00 g	0 h
0,0	4,0	2,93 tu	8,65 pq	0,00 e	0,00 g	0 h
0,0	8,0	2,85 u	7,98 q	0,00 e	0,00 g	0 h
0,25	0,0	4,85 ghij	12,00 k-o	0,00 e	0,00 g	0 h
0,25	0,1	4,05 mnop	11,73 k-p	0,00 e	0,00 g	0 h
0,25	0,5	3,73 opq	10,53 l-q	0,00 e	0,00 g	0 h
0,25	1,0	3,60 pqrs	10,37 l-q	0,00 e	0,00 g	0 h
0,25	2,0	3,48 qrst	9,75 nopq	0,00 e	0,00 g	0 h
0,25	4,0	3,10 stu	8,78 opq	0,00 e	0,00 g	0 h
0,25	8,0	2,88 u	8,04 q	0,00 e	0,00 g	0 h
0,5	0,0	5,25 efg	17,10 j	0,00 e	0,15 g	15 fg
0,5	0,1	5,05 fghi	16,85 j	0,00 e	0,10 g	10 gh
0,5	0,5	4,64 h-l	13,06 kl	0,00 e	0,05 g	5 gh
0,5	1,0	4,44 j-n	12,78 klm	0,00 e	0,00 g	0 h
0,5	2,0	3,70 opqr	10,38 l-q	0,00 e	0,00 g	0 h
0,5	4,0	3,40 q-u	9,00 opq	0,00 e	0,00 g	0 h
0,5	8,0	2,98 tu	8,19 q	0,00 e	0,00 g	0 h
1,0	0,0	5,60 cde	26,50 h	0,00 e	1,00 e	65 c
1,0	0,1	5,52 def	22,82 i	0,00 e	0,50 f	40 e
1,0	0,5	4,75 g-k	16,18 j	0,00 e	0,30 fg	30 f
1,0	1,0	4,56 h-m	13,81 k	0,00 e	0,00 g	0 h
1,0	2,0	4,49 i-n	10,48 l-q	0,00 e	0,00 g	0 h
1,0	4,0	4,03 nop	9,08 opq	0,00 e	0,00 g	0 h
1,0	8,0	3,05 stu	8,27 q	11,50 d	0,00 g	0 h
2,0	0,0	5,72 cde	35,60 ef	0,00 e	1,30 d	85 ab
2,0	0,1	5,64 cde	30,67 g	0,00 e	1,10 e	55 d
2,0	0,5	5,50 def	24,22 i	0,00 e	0,50 f	50 d
2,0	1,0	4,72 g-k	17,10 j	0,00 e	0,10 g	10 gh
2,0	2,0	4,59 h-l	15,75 j	0,00 e	0,00 g	0 h
2,0	4,0	4,11 lmno	9,22 opq	0,00 e	0,00 g	0 h
2,0	8,0	3,10 stu	8,33 q	55,97 b	0,00 g	0 h
4,0	0,0	6,25 ab	45,60 c	0,00 e	2,00 b	100 a
4,0	0,1	5,95 bcd	40,50 d	0,00 e	1,80 bc	100 a
4,0	0,5	5,75 cde	34,21 f	0,00 e	1,60 c	100 a
4,0	1,0	4,77 g-k	28,06 h	0,00 e	1,40 d	100 a
4,0	2,0	4,73 g-k	27,45 h	0,00 e	1,00 e	80 b
4,0	4,0	4,15 lmno	12,57 klmn	41,28 c	0,00 g	0 h
4,0	8,0	3,18 rstu	8,85 opq	66,15 a	0,00 g	0 h
8,0	0,0	6,50 a	50,80 a	0,00 e	2,30 a	100 a
8,0	0,1	6,10 abc	48,56 b	0,00 e	2,00 b	100 a
8,0	0,5	5,86 bcd	37,07 e	0,00 e	1,80 bc	100 a
8,0	1,0	5,10 fgh	34,00 f	0,00 e	1,65 c	100 a
8,0	2,0	4,88 ghij	27,75 h	0,00 e	1,05 e	85 ab
8,0	4,0	4,26 klmn	13,60 k	0,00 e	0,50 f	20 fg
8,0	8,0	3,20 q-u	8,98 opq	0,00 e	0,00 g	0 h

a-u: Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level.

APPENDIX 3.2 - The effect of different concentration ratios of kinetin and indole-3-butyric acid (IBA) on shoot elongation, total shoot mass, callus mass, leaf expansion and percentages of explants with expanded leaves derived from shoot apices of US 1-9 cultured *in vitro*

Treatment (mg/ℓ) KIN : IBA		Elongation (mm)	Shoot mass (mg)	Callus mass (mg)	Number of expanded leaves	% explants with expanded leaves
0,0	0,0	3,35 fghi	9,80 de	0,00 d	0,00 d	0 f
0,0	0,1	3,06 ghi	9,53 de	0,00 d	0,00 d	0 f
0,0	0,5	3,02 ghi	9,10 de	0,00 d	0,00 d	0 f
0,0	1,0	3,00 ghi	9,05 de	0,00 d	0,00 d	0 f
0,0	2,0	2,88 hi	9,00 de	0,00 d	0,00 d	0 f
0,0	4,0	2,50 i	8,76 de	0,00 d	0,00 d	0 f
0,0	8,0	2,48 i	8,00 e	0,00 d	0,00 d	0 f
0,25	0,0	4,38 d-i	13,98 de	0,00 d	0,00 d	0 f
0,25	0,1	4,18 d-i	12,00 de	0,00 d	0,00 d	0 f
0,25	0,5	4,08 d-i	11,58 de	0,00 d	0,00 d	0 f
0,25	1,0	4,05 d-i	11,55 de	0,00 d	0,00 d	0 f
0,25	2,0	3,82 d-i	10,61 de	0,00 d	0,00 d	0 f
0,25	4,0	3,65 e-i	9,88 de	0,00 d	0,00 d	0 f
0,25	8,0	3,02 ghi	8,32 e	0,00 d	0,00 d	0 f
0,5	0,0	4,60 d-i	19,66 de	0,00 d	0,20 d	20 e
0,5	0,1	4,40 d-i	13,96 de	0,00 d	0,00 d	0 f
0,5	0,5	4,38 d-i	13,50 de	0,00 d	0,00 d	0 f
0,5	1,0	4,22 d-i	12,80 de	0,00 d	0,00 d	0 f
0,5	2,0	4,16 d-i	10,80 de	0,00 d	0,00 d	0 f
0,5	4,0	4,08 d-i	10,26 de	0,00 d	0,00 d	0 f
0,5	8,0	3,21 ghi	8,96 de	50,40 d	0,00 d	0 f
1,0	0,0	4,95 c-i	28,54 de	0,00 d	1,10 cd	75 b
1,0	0,1	4,58 d-i	16,76 de	0,00 d	0,00 d	0 f
1,0	0,5	4,52 d-i	16,38 de	0,00 d	0,00 d	0 f
1,0	1,0	4,38 d-i	14,98 de	0,00 d	0,00 d	0 f
1,0	2,0	4,21 d-i	12,50 de	133,28 cd	0,00 d	0 f
1,0	4,0	4,16 d-i	11,85 de	252,68 c	0,00 d	0 f
1,0	8,0	3,28 ghi	9,27 de	266,82 c	0,00 d	0 f
2,0	0,0	5,83 b-f	45,88 cde	0,00 d	1,80 bc	100 a
2,0	0,1	5,21 c-h	39,63 cde	0,00 d	1,20 cd	80 b
2,0	0,5	4,80 c-i	21,62 de	0,00 d	1,00 cd	60 c
2,0	1,0	4,62 d-i	15,02 de	128,39 cd	0,00 d	0 f
2,0	2,0	4,48 d-i	13,60 de	153,08 cd	0,00 d	0 f
2,0	4,0	4,25 d-i	12,12 de	513,62 b	0,00 d	0 f
2,0	8,0	4,16 d-i	10,05 de	1 240,68 a	0,00 d	0 f
4,0	0,0	7,35 b	83,63 c	0,00 d	2,30 b	100 a
4,0	0,1	6,25 bcd	70,62 cd	0,00 d	1,60 bc	90 a
4,0	0,5	5,95 bcde	42,65 cde	0,00 d	1,00 cd	80 b
4,0	1,0	5,56 b-g	38,38 cde	183,62 cd	0,00 d	0 f
4,0	2,0	5,24 c-h	30,08 de	256,04 c	0,00 d	0 f
4,0	4,0	4,45 d-i	21,62 de	1 195,76 a	0,00 d	0 f
4,0	8,0	4,21 d-i	10,61 de	195,68 cd	0,00 d	0 f
8,0	0,0	8,75 a	248,52 a	0,00 d	3,20 a	100 a
8,0	0,1	6,92 bc	148,52 b	0,00 d	2,30 b	100 a
8,0	0,5	6,36 bcd	83,48 c	0,00 d	1,80 bc	100 a
8,0	1,0	5,98 bcde	52,62 cde	0,00 d	1,00 cd	50 d
8,0	2,0	5,38 b-h	33,62 de	0,00 d	0,00 d	0 f
8,0	4,0	4,92 c-i	31,91 de	0,00 d	0,00 d	0 f
8,0	8,0	4,38 d-i	25,42 de	0,00 d	0,00 d	0 f

a-i: Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level.

APPENDIX 4.1 - Effects of increasing concentrations of kinetin on shoot elongation, total shoot mass, number of proliferated shoots and percentages of explants with proliferated shoots derived from shoots apices of (1) Chenin blanc and (2) US 1-9 cultured *in vitro*

Treatment (mg/ℓ)	Elongation (mm)		Shoot mass (mg)		Number of proliferated shoots		% explants with proliferated shoots	
Kinetin	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Control	2,95 f	2,64 i	8,45 f	7,54 d	0,00 b	0,00 c	0 b	0 c
0,1	3,23 f	3,11 h	9,98 f	9,82 d	0,00 b	0,00 c	0 b	0 c
0,25	3,94 e	4,03 g	11 06 f	12,67 d	0,00 b	0,00 c	0 b	0 c
0,5	4,89 d	4,20 fg	17,95 e	15,88 d	0,00 b	0,00 c	0 b	0 c
1	5,22 cd	4,51 f	25,40 d	20,95 d	0,00 b	0,00 c	0 b	0 c
2	5,29 c	5,19 e	37,82 c	32,85 d	0,00 b	0,00 c	0 b	0 c
5	6,07 b	6,38 d	40,94 c	70,22 c	0,00 b	0,00 c	0 b	0 c
10	6,29 b	7,10 c	45,02 b	110,10 b	0,00 b	0,10 bc	0 b	10 bc
15	6,88 a	7,76 b	49,31 a	123,62 b	0,10 ab	0,20 b	10 ab	20 b
20	7,10 a	8,80 a	49,92 a	195,67 a	0,20 a	0,80 a	20 a	50 a

a-i: Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level of significance.

APPENDIX 4.2 - Effects of increasing concentrations of 6-benzylaminopurine (BAP) on shoot elongation, total shoot mass, number of proliferated shoots and percentages of explants with proliferated shoots derived from shoot apices of (1) Chenin blanc and (2) US 1-9 cultured *in vitro*

Treatment (mg/ℓ)	Elongation (mm)		Shoot mass (mg)		Number of proliferated shoots		% explants with proliferated shoots	
BAP	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Control	2,85 e	2,80 f	8,60 d	8,40 e	0,00 b	0,00 b	0 b	0 c
0,1	5,08 d	6,84 d	20,08 d	28,55 de	0,00 b	0,00 b	0 b	0 c
0,25	6,55 c	7,62 d	52,62 c	60,00 cde	0,00 b	0,00 b	0 b	0 c
0,5	6,69 c	11,06 c	65,00 c	75,84 cd	0,00 b	0,00 b	0 b	0 c
1	10,19 b	12,80 b	125,31 b	142,54 b	0,00 b	1,00 b	0 b	60 b
2	14,09 a	16,28 a	210,00 a	381,87 a	2,00 a	2,40 a	90 a	100 a
5	2,90 e	5,46 e	19,39 d	88,63 c	0,00 b	0,00 b	0 b	0 c
10	2,75 e	3,40 f	15,40 d	22,35 e	0,00 b	0,00 b	0 b	0 c
15	2,60 e	3,10 f	10,00 d	18,61 e	0,00 b	0,00 b	0 b	0 c
20	2,50 e	2,85 f	8,30 d	11,00 e	0,00 b	0,00 b	0 b	0 c

a-f: Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level of significance.

APPENDIX 4.3 - Effects of increasing concentrations of zeatin on shoot elongation, total shoot mass, number of proliferated shoots and percentages of explants with proliferated shoots derived from shoot apices of (1) Chenin blanc and (2) US 1-9 cultured *in vitro*

Treatment (mg/ℓ)	Elongation (mm)		Shoot mass (mg)		Number of proliferated shoots		% explants with proliferated shoots	
Zeatin	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Control	2,80 e	2,70 d	8,70 f	8,50 e	0,00 b	0,00 c	0 c	0 d
0,1	5,80 de	4,95 cd	43,80 ef	39,80 de	0,00 b	0,00 c	0 c	0 d
0,25	7,10 de	6,00 cd	55,32 ef	52,16 de	0,00 b	0,00 c	0 c	0 d
0,5	8,70 cd	12,40 b	73,22 e	76,82 d	0,00 b	0,00 c	0 c	0 d
1	9,50 cd	12,90 b	88,39 e	92,66 d	0,00 b	0,00 c	0 c	0 d
2	9,80 cd	13,50 b	192,61 d	205,30 c	0,00 b	0,20 bc	0 c	20 c
5	12,10 bc	20,70 a	262,81 c	295,32 b	0,20 b	1,00 abc	20 b	80 b
10	15,60 ab	22,60 a	382,68 b	458,86 a	1,20 a	1,30 ab	100 a	100 a
15	19,90 a	26,90 a	489,55 a	502,35 a	1,50 a	2,00 a	100 a	100 a
20	10,50 cd	10,75 bc	293,73 c	298,00 b	0,10 b	0,20 bc	10 bc	20 c

a-f: Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level of significance.

APPENDIX 4.4 - Effects of increasing concentrations of zeatin riboside (ZR) on shoot elongation, total shoot mass, number of proliferated shoots and percentages of explants with proliferated shoots derived from shoot apices of (1) Chenin blanc and (2) US 1-9 cultured *in vitro*

Treatment (mg/ℓ)	Elongation (mm)		Shoot mass (mg)		Number of proliferated shoots		% explants with proliferated shoots	
ZR	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Control	2,90 e	2,75 g	8,70 f	8,50 g	0,00 b	0,00 b	0 b	0 c
0,1	6,20 de	6,90 fg	23,63 f	29,19 fg	0,00 b	0,00 b	0 b	0 c
0,25	6,70 d	9,00 ef	63,27 e	73,88 ef	0,00 b	0,00 b	0 b	0 c
0,5	11,85 c	12,50 de	71,62 e	78,72 ef	0,00 b	0,00 b	0 b	0 c
1	12,10 c	13,12 cde	75,73 e	86,38 e	0,00 b	0,00 b	0 b	0 c
2	18,26 b	19,15 b	240,48 c	263,24 c	0,00 b	1,00 b	0 b	80 ab
5	18,95 b	20,10 b	332,68 b	364,32 b	1,00 b	1,10 b	100 a	100 a
10	32,60 a	33,72 a	749,32 a	785,68 a	2,80 a	2,90 a	100 a	100 a
15	13,82 c	14,20 cd	149,25 d	192,68 d	0,00 b	0,80 b	0 b	60 b
20	7,00 d	7,22 f	82,76 e	102,54 e	0,00 b	0,00 b	0 b	0 c

a-g: Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level of significance.

APPENDIX 5.1 - The effect of different concentration ratios of 6-benzylaminopurine (BAP) and 1-naphthaleneacetic acid (NAA) on shoot elongation, total shoot mass, callus mass, shoot proliferation and percentages of explants with proliferated shoots derived from shoot apices of Chenin blanc cultured in vitro

Treatment (mg/ℓ) BAP	: NAA	Elongation (mm)	Shoot mass (mg)	Callus mass (mg)	Number of prolife-rated shoots	% of explants with pro-liferated shoots
0,0	0,0	3,30 d	10,60 e	0,00 e	0,00 c	0 c
0,0	0,1	3,20 d	9,02 e	0,00 e	0,00 c	0 c
0,0	1,0	3,00 d	6,85 e	7,22 de	0,00 c	0 c
0,0	2,0	2,80 d	6,32 e	11,84 de	0,00 c	0 c
0,0	5,0	2,65 d	5,27 e	14,08 cde	0,00 c	0 c
0,5	0,0	9,10 c	74,62 de	0,00 e	0,00 c	0 c
0,5	0,1	8,90 c	82,87 de	0,00 e	0,00 c	0 c
0,5	1,0	3,90 d	15,62 e	7,86 de	0,00 c	0 c
0,5	2,0	2,95 d	6,53 e	19,42 cde	0,00 c	0 c
0,5	5,0	2,70 d	5,50 e	29,88 c	0,00 c	0 c
1,0	0,0	12,60 b	166,38 c	0,00 e	0,00 c	0 c
1,0	0,1	11,60 bc	183,42 c	0,00 e	1,00 bc	60 b
1,0	1,0	4,95 d	16,76 e	9,52 de	0,00 c	0 c
1,0	2,0	3,15 d	6,90 e	22,63 cd	0,00 c	0 c
1,0	5,0	2,85 d	5,73 e	83,62 a	0,00 c	0 c
2,0	0,0	19,20 a	436,28 a	0,00 e	2,40 a	90 a
2,0	0,1	12,90 b	298,46 b	0,00 e	1,80 ab	80 a
2,0	1,0	5,20 d	32,80 e	9,80 de	0,00 c	0 c
2,0	2,0	5,05 d	18,95 e	23,42 cd	0,00 c	0 c
2,0	5,0	4,80 d	17,85 e	42,25 b	0,00 c	0 c
3,5	0,0	9,80 c	123,85 d	0,00 e	0,00 c	0 c
3,5	0,1	6,40 d	48,46 e	0,00 e	0,00 c	0 c
3,5	1,0	4,60 d	22,52 e	6,23 de	0,00 c	0 c
3,5	2,0	4,20 d	15,45 e	11,82 de	0,00 c	0 c
3,5	5,0	4,00 d	12,13 e	23,85 cd	0,00 c	0 c

a - e: Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level.

APPENDIX 5.2 - The effect of different concentration ratios of 6-benzylaminopurine (BAP) and 1-naphthaleneacetic acid (NAA) on shoot elongation, total shoot mass, callus mass, shoot proliferation and percentages of explants with proliferated shoots derived from shoot apices of US 1-9 cultured in vitro

Treatment (mg/ℓ) BAP	NAA	Elongation (mm)	Shoot mass (mg)	Callus mass (mg)	Number of prolife-rated shoots	% of explants with pro-liferated shoots
0,0	0,0	3,20 efg	9,78 e	0,00 f	0,00 b	0 e
0,0	0,1	3,10 efg	8,83 e	0,00 f	0,00 b	0 e
0,0	1,0	2,90 fg	7,23 e	5,16 f	0,00 b	0 e
0,0	2,0	2,70 g	6,67 e	13,82 f	0,00 b	0 e
0,0	5,0	2,50 g	5,76 e	38,63 ef	0,00 b	0 e
0,5	0,0	18,70 b	105,64 cd	0,00 f	0,70 b	30 d
0,5	0,1	9,20 de	93,68 cde	0,00 f	0,00 b	0 e
0,5	1,0	7,20 defg	78,68 cde	14,86 f	0,00 b	0 e
0,5	2,0	4,90 defg	18,25 de	96,38 d	0,00 b	0 e
0,5	5,0	3,00 efg	6,82 e	51,72 ef	0,00 b	0 e
1,0	0,0	20,80 b	202,84 b	0,00 f	3,40 a	80 b
1,0	0,1	13,60 c	103,86 cd	0,00 f	0,50 b	40 c
1,0	1,0	8,20 defg	92,50 cde	18,28 f	0,00 b	0 e
1,0	2,0	5,30 defg	19,86 de	215,88 b	0,00 b	0 e
1,0	5,0	4,00 defg	8,83 e	75,88 e	0,00 b	0 e
2,0	0,0	32,60 a	532,82 a	0,00 f	4,80 a	100 a
2,0	0,1	21,70 b	489,32 a	0,00 f	4,10 a	100 a
2,0	1,0	9,00 def	97,25 cde	163,28 c	0,00 b	0 e
2,0	2,0	6,10 defg	20,04 de	292,56 a	0,00 b	0 e
2,0	5,0	4,20 defg	18,05 de	183,85 bc	0,00 b	0 e
3,5	0,0	9,90 d	126,38 c	0,00 f	0,00 b	0 e
3,5	0,1	7,80 defg	88,68 cde	0,00 f	0,00 b	0 e
3,5	1,0	5,40 defg	39,62 de	23,48 f	0,00 b	0 e
3,5	2,0	5,20 defg	18,28 de	28,28 f	0,00 b	0 e
3,5	5,0	4,10 defg	13,26 e	24,29 f	0,00 b	0 e

a - g: Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level.

APPENDIX 6.1 - The effect of different concentration ratios of zeatin riboside (ZR) and 1-naphthaleneacetic acid (NAA) on shoot elongation, total shoot mass, callus mass, shoot proliferation and percentages of explants with proliferated shoots derived from shoot apices of Chenin blanc cultered in vitro

Treatment (mg/ℓ) ZR : NAA		Elongation (mm)	Shoot mass (mg)	Callus mass (mg)	Number of prolife-rated shoots	% of explants with pro-liferated shoots
0,0	0,0	3,35 d	10,76 d	0,00 e	0,00 c	0 c
0,0	0,1	3,15 d	8,95 d	0,00 e	0,00 c	0 c
0,0	1,0	3,10 d	6,83 d	7,92 e	0,00 c	0 c
0,0	2,0	2,90 d	6,28 d	12,57 e	0,00 c	0 c
0,0	5,0	2,75 d	5,52 d	13,82 e	0,00 c	0 c
1,0	0,0	13,90 c	163,48 d	0,00 e	0,00 c	0 c
1,0	0,1	9,65 cd	32,68 d	2,55 e	0,00 c	0 c
1,0	1,0	4,40 d	18,72 d	104,96 de	0,00 c	0 c
1,0	2,0	4,10 d	11,95 d	116,83 de	0,00 c	0 c
1,0	5,0	3,05 d	8,03 d	202,52 cde	0,00 c	0 c
2,0	0,0	22,60 b	244,16 d	0,00 e	0,00 c	0 c
2,0	0,1	21,50 b	233,68 d	10,46 e	0,00 c	0 c
2,0	1,0	5,30 d	25,52 d	115,52 de	0,00 c	0 c
2,0	2,0	4,90 d	22,82 d	186,32 cde	0,00 c	0 c
2,0	5,0	3,35 d	11,36 d	332,85 bc	0,00 c	0 c
5,0	0,0	24,85 b	520,66 c	0,00 e	1,10 c	100 a
5,0	0,1	24,30 b	550,00 c	12,11 e	3,40 b	80 b
5,0	1,0	8,25 cd	27,92 d	162,88 cde	0,00 c	0 c
5,0	2,0	6,25 d	23,65 d	193,82 cde	0,00 c	0 c
5,0	5,0	4,20 d	12,59 d	382,55 b	0,00 c	0 c
10,0	0,0	35,80 a	962,87 b	0,00 e	3,20 b	100 a
10,0	0,1	34,90 a	1 248,63 a	13,85 e	7,00 a	100 a
10,0	1,0	8,60 cd	32,82 d	163,08 cde	0,00 c	0 c
10,0	2,0	7,40 d	31,55 d	302,65 bcd	0,00 c	0 c
10,0	5,0	4,80 d	13,26 d	552,83 a	0,00 c	0 c

a - e: Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level.

APPENDIX 6.2 - The effect of different concentration ratios of zeatin riboside (ZR) and 1-naphthaleneacetic acid (NAA) on shoot elongation, total shoot mass, callus mass, shoot proliferation and percentages of explants with proliferated shoots derived from shoot apices of US 1-9 cultured in vitro

Treatment (mg/ℓ) ZR : NAA		Elongation (mm)	Shoot mass (mg)	Callus mass (mg)	Number of prolife-rated shoots	% of explants with pro-liferated shoots
0,0	0,0	3,30 f	9,85 d	0,00 c	0,00 c	0 c
0,0	0,1	3,05 f	8,72 d	0,00 c	0,00 c	0 c
0,0	1,0	2,80 f	7,38 d	5,34 c	0,00 c	0 c
0,0	2,0	2,60 f	6,85 d	15,56 c	0,00 c	0 c
0,0	5,0	2,50 f	5,87 d	43,28 c	0,00 c	0 c
1,0	0,0	25,30 cde	228,32 d	0,00 c	0,00 c	0 c
1,0	0,1	16,40 def	212,67 d	18,63 c	0,00 c	0 c
1,0	1,0	3,80 f	19,56 d	1 025,68 b	0,00 c	0 c
1,0	2,0	3,20 f	12,36 d	1 895,38 a	0,00 c	0 c
1,0	5,0	3,10 f	9,19 d	96,35 c	0,00 c	0 c
2,0	0,0	34,70 bc	495,68 c	0,00 c	1,30 bc	80 b
2,0	0,1	26,30 cde	238,52 d	38,96 c	0,00 c	0 c
2,0	1,0	6,50 f	28,46 d	1 892,84 a	0,00 c	0 c
2,0	2,0	5,50 f	23,32 d	1 965,96 a	0,00 c	0 c
2,0	5,0	3,90 f	18,68 d	164,18 c	0,00 c	0 c
5,0	0,0	40,60 abc	685,38 b	0,00 c	1,80 bc	100 a
5,0	0,1	30,80 bcd	573,29 bc	42,77 c	2,00 b	80 b
5,0	1,0	10,60 ef	80,63 d	1 973,92 a	0,00 c	0 c
5,0	2,0	7,70 f	43,28 d	2 149,36 a	0,00 c	0 c
5,0	5,0	5,20 f	22,18 d	253,55 c	0,00 c	0 c
10,0	0,0	52,50 a	988,52 a	0,00 c	3,40 a	100 a
10,0	0,1	42,60 ab	945,82 a	74,22 c	3,60 a	100 a
10,0	1,0	11,00 ef	142,82 d	142,68 c	0,00 c	0 c
10,0	2,0	8,00 f	55,36 d	303,92 c	0,00 c	0 c
10,0	5,0	5,90 f	30,07 d	283,48 c	0,00 c	0 c

a - f: Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level.

APPENDIX 7.1 - The effect of different concentration ratios of zeatin riboside (ZR) and gibberellic acid (GA_3) on shoot elongation, total shoot mass, leaf expansion and percentages of explants with expanded leaves derived from shoot apices of Chenin blanc cultured *in vitro*

Treatment (mg/ℓ) ZR : GA_3		Elongation (mm)	Shoot mass (mg)	Number of expanded leaves	% explants with expanded leaves
0,0	0,0	2,30 i	4,21 h	0,00 j	0 e
0,0	0,5	3,70 hi	7,75 h	0,00 j	0 e
0,0	1,0	5,60 ghi	10,47 h	0,00 j	0 e
0,0	2,0	5,90 fghi	12,75 h	0,00 j	0 e
0,0	5,0	7,80 fghi	14,26 h	0,40 ij	40 cd
0,0	10,0	8,20 e-i	14,98 h	0,60 ij	60 b
1,0	0,0	6,50 fghi	18,63 h	0,40 ij	30 d
1,0	0,5	8,00 fghi	28,62 h	0,80 ij	50 bc
1,0	1,0	9,80 d-h	63,10 gh	1,00 hij	60 b
1,0	2,0	10,70 c-g	72,52 fgh	1,20 ghi	80 a
1,0	5,0	13,00 cdef	113,06 efgh	2,00 efg	100 a
1,0	10,0	16,20 abcd	164,28 defg	2,40 def	100 a
2,0	0,0	8,60 e-i	80,28 fgh	1,00 hij	80 a
2,0	0,5	11,20 c-g	85,00 fgh	1,20 ghi	80 a
2,0	1,0	11,80 c-g	193,24 cdef	2,80 cde	100 a
2,0	2,0	16,80 abc	210,76 cde	3,00 bcde	100 a
2,0	5,0	19,80 ab	253,80 cd	3,00 bcde	100 a
2,0	10,0	20,00 ab	273,67 cd	3,40 abcd	100 a
5,0	0,0	9,80 d-h	110,28 efgh	1,80 fgh	80 a
5,0	0,5	11,80 c-g	165,39 defg	2,40 def	90 a
5,0	1,0	12,30 c-g	210,35 cde	3,00 bcde	100 a
5,0	2,0	17,10 abc	280,63 cd	3,10 bcd	100 a
5,0	5,0	20,30 ab	295,88 c	3,60 abc	100 a
5,0	10,0	21,20 ab	303,23 c	3,60 abc	100 a
10,0	0,0	11,00 c-g	182,32 c-g	2,80 cde	100 a
10,0	0,5	15,00 bcde	210,50 cde	3,80 abc	100 a
10,0	1,0	19,60 ab	410,20 b	4,00 ab	100 a
10,0	2,0	20,30 ab	560,75 a	4,00 ab	100 a
10,0	5,0	22,30 a	592,85 a	4,00 ab	100 a
10,0	10,0	22,50 a	620,28 a	4,40 a	100 a

a-j: Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level of significance.

APPENDIX 7.2 - The effect of different concentration ratios of zeatin riboside (ZR) and gibberellic acid (GA_3) on shoot elongation, total shoot mass, leaf expansion and percentages of explants with expanded leaves derived from shoot apices of US 1-9 cultured *in vitro*

Treatment (mg/ℓ) ZR : GA_3		Elongation (mm)	Shoot mass (mg)	Number of expanded leaves	% explants with expanded leaves
0,0	0,0	2,00 g	4,05 g	0,00 d	0 d
0,0	0,5	3,20 fg	7,12 g	0,00 d	0 d
0,0	1,0	4,50 efg	9,82 g	0,00 d	0 d
0,0	2,0	4,70 defg	10,69 g	0,00 d	0 d
0,0	5,0	5,10 defg	12,86 g	0,20 d	10 d
0,0	10,0	5,60 defg	13,59 g	0,40 cd	40 c
1,0	0,0	7,60 defg	33,48 g	0,50 cd	40 c
1,0	0,5	7,70 defg	36,60 g	0,80 bcd	60 b
1,0	1,0	10,20 c-f	82,68 f	1,60 abcd	80 a
1,0	2,0	11,80 b-e	98,68 ef	1,80 abcd	90 a
1,0	5,0	12,10 b-e	132,44 de	2,30 abcd	100 a
1,0	10,0	15,60 abcd	140,29 de	2,40 abcd	100 a
2,0	0,0	9,80 c-f	101,42 ef	1,80 abcd	90 a
2,0	0,5	10,60 c-f	119,25 def	2,00 abcd	100 a
2,0	1,0	12,40 b-e	195,88 bc	2,70 abc	100 a
2,0	2,0	14,00 b-e	199,46 bc	3,00 ab	100 a
2,0	5,0	19,70 abc	212,72 b	3,00 ab	100 a
2,0	10,0	21,20 ab	253,98 ab	3,20 ab	100 a
5,0	0,0	10,00 c-f	135,62 de	2,00 abcd	100 a
5,0	0,5	11,30 b-e	160,49 cd	2,40 abcd	100 a
5,0	1,0	13,00 b-e	232,48 ab	3,00 ab	100 a
5,0	2,0	14,80 a-e	250,42 ab	3,40 a	100 a
5,0	5,0	20,30 abc	253,82 ab	3,50 a	100 a
5,0	10,0	22,80 a	283,88 a	3,50 a	100 a
10,0	0,0	11,20 b-e	198,56 bc	3,00 ab	100 a
10,0	0,5	12,00 b-e	205,69 bc	3,80 a	100 a
10,0	1,0	13,10 b-e	268,45 a	3,80 a	100 a
10,0	2,0	12,10 b-e	248,46 ab	3,60 a	100 a
10,0	5,0	11,60 b-e	238,36 ab	3,50 a	100 a
10,0	10,0	10,50 c-f	202,58 bc	2,20 abcd	100 a

a-g: Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level of significance.

APPENDIX 8 - The effect of different concentration ratios of 6-benzylaminopurine (BAP) and zeatin riboside (ZR) on elongation, total shoot mass, shoot proliferation and percentages of explants with proliferated shoots derived from shoot apices of Chenin blanc cultured in vitro

Treatment (mg/ℓ) BAP : ZR	Elongation (mm)	Shoot mass (mg)	Number of proliferated shoots	% of explants with proliferated shoots
0,0 0,0	3,22 f	10,60 k	0,00 f	0 b
0,0 1,0	14,27 d	185,50 ijk	0,00 f	0 b
0,0 2,0	22,72 bc	245,80 hijk	0,00 f	0 b
0,0 5,0	23,02 bc	701,00 defg	1,20 ef	100 a
0,0 10,0	36,24 a	853,90 cde	3,40 def	100 a
0,5 0,0	8,95 e	77,80 jk	0,00 f	0 b
0,5 1,0	21,15 bc	344,20 hijk	2,00 def	90 a
0,5 2,0	23,83 bc	433,00 ghij	2,10 def	100 a
0,5 5,0	24,55 bc	881,70 cde	3,10 def	100 a
0,5 10,0	26,08 b	1 107,00 abc	6,20 cd	100 a
1,0 0,0	12,11 de	170,20 ijk	0,00 f	0 b
1,0 1,0	21,85 bc	382,60 g-k	2,30 def	100 a
1,0 2,0	23,95 bc	587,40 efgh	4,40 cdef	100 a
1,0 5,0	25,00 bc	1 012,50 bcd	7,65 bc	100 a
1,0 10,0	23,85 bc	1 248,70 ab	9,65 b	100 a
2,0 0,0	19,14 c	430,50 ghij	2,30 def	90 a
2,0 1,0	22,00 bc	501,30 fghi	3,00 def	100 a
2,0 2,0	25,13 bc	1 343,50 a	14,30 a	100 a
2,0 5,0	23,95 bc	781,50 def	5,30 cde	100 a
2,0 10,0	13,33 de	248,30 hijk	2,55 def	100 a
5,0 0,0	3,29 f	25,20 k	0,00 f	0 b
5,0 1,0	3,33 f	27,90 k	0,00 f	0 b
5,0 2,0	3,60 f	30,50 k	0,00 f	0 b
5,0 5,0	3,70 f	32,10 k	0,00 f	0 b
5,0 10,0	3,80 f	32,62 k	0,00 f	0 b

a-k: Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level of significance.

OPSOMMING

Veertien verskillende kallusmetodes is onder laboratoriumtoestande uitgetoets t.o.v. die kallusvormingsvermoë van vier onderstokcultivars. Duidelike verskille is tussen die metodes waargeneem. Aktiewe kallusontwikkeling oor 'n kort periode (14 dae) is in die geval van plastieksakke, uitgevoer met klam vloeipapier, waargeneem. Laasgenoemde het duidelik as die beste metode uitgestaan.

Duidelike verskille t.o.v. kallusvormingsvermoë is tussen vier bekende onderstokcultivars en drie nuwe onderstokkruisings onder laboratoriumtoestande waargeneem. In alle gevalle kon cultivars gerangskik word van beste tot swakste kallusvormingsvermoë. Die betrokke onderstokcultivars- en kruisings is ook aan kallusvormingstudies onder buitelugtoestande (herfs) onderwerp. Duidelike variasies t.o.v. kallusvormingsvermoë is aan die lig gebring en groeperings van beste tot swakste saamgestel.

In ondersoekings t.o.v. moontlike kallusstimulante is gevind dat 'n sekere groeireguleerder-mengsel sterk kallusstimulerend opgetree het by winterlote van Salt Creek. Inhibering van kallusvorming het voorgekom by sekere groeireguleerders, veral by hoër konsentrasies en langer behandelingstye.

Die uittoets van verskeie swam- en bakteriedoders op die kallusvormingsvermoë van winterlote van 99R. het aan die lig gebring dat kallusvorming ernstig benadeel kan word by sekere behandelings. Laasgenoemde invloed is hoofsaaklik by hoë konsentrasies en lang indompelingsperiodes (2 uur) waargeneem.

BIOLETTI, F.T., 1921. Propagation of vines. Univ. of Cal. Circular No. 225.

BIOLETTI, F.T., FLOSSFEDER, F.C.H. & WAY, A.E., 1921. Phylloxera-resistant stocks. Univ. of Cal. Pub. Bulletin No. 331.

BLOCH, R., 1952. Wound healing in higher plants. Bot. Rev. 18, 655 - 675.

BONNER, J. & ENGLISH, J., 1938. A chemical and physiological study of traumatin, a plant wound hormone. Plant Physiol. 13, 331 - 348.

BONNET, L.O., 1925. Phylloxera resistant vineyards. Univ. of Cal. Circular No. 288.

BOSIAN, G., 1938. Oelemulsionen und Wushsstoffe in ihrer praktischen Bedeutung für Pfropfrebenbau und Stecklingsvermehrung. Wein und Rebe 20, 299 - 309.

BROWN, A.B., 1937. Activity of the vascular cambium in relation to wounding in the Balsam poplar. Canad. Jour. Res. 15, 7 - 31.

CLELAND, R. & BONNER, J., 1956. The residual effect of auxin on the cell wall. Plant Physiol. 31, 350 - 354.

CORMACK, R.G.H., 1965. The effects of calcium ions and pH on the development of callus tissue on stem cuttings of Balsam poplar. Canad. Jour. Bot. 43, 75 - 83.

CORMACK, R.G.H. & LEMAY, P.L., 1966. A further study of callus tissue development on stem cuttings of Balsam poplar. Canad. Jour. Bot. 44, 47 - 50.

DAVIS, E.A., 1949. Effects of several growth-regulators on wound healing of Sugar Maple. Bot. Gaz. 111, 69 - 77.

DE ROPP, R.S., 1947. The growth-promoting and tumefacient factors of bacteria-free crown-gall tissues. Amer. Jour. Bot. 34, 248 - 261.

DE ROPP, R.S., 1950. The comparative growth promoting action of Indole-3-acetic acid and Agrobacterium tumefaciens. Am. Jour. Bot. 37, 352 - 363.

DEVLIN, R.M., 1967. Plant Physiology. Reinhold Publishing Corp. New York.

DORAN, W.L., 1953. Effects of treatint cuttings of woody plants with both a root-inducing substance and a fungicide. Proc. Amer. Soc. Hort. Sci. 60, 487 - 491.

DUGGER, W.M., HUMPHREYS, T.E. & CALHOUN, B., 1957. Influence of captan on the metabolism of pea and corn seedlings. Plant Phys. 32, (suppl.) vii.

EIFERT, J., BáLó, E., BáLó, S., EIFERT, A. & PáNCZéL, M., 1966. Physiologische Erkenntnisse und agrotechnische Verfahren bei Grossbetrieben. Weinberg und Keller 13, 107 - 120.

EIFERT, J., BáLó, E. & EIFERT, A., 1970. Über technische Probleme der Lagerung und das Transportes von Veredlungsholz unter besonderer Berücksichtigung des Wasserhaushaltes und der Rebschultechnik. Weinberg und Keller 17, 545 - 560.

EIFERT, J. & EIFERT, A., 1962. Wirkung einiger IES-Vorstufen auf die Kallusbildung der Weinrebe während der Winterruhe. Naturwiss. 49, 522.

EIFERT, J. & EIFERT, A., 1965. Beobachtungen über die kallusfördernde Wirkung von Chinosol. Wein-Wissenschaft 21, 325 - 328.

ERMAKOV, B.S. & GREVIN, J.A., 1966. Substrate, illumination and rooting of cuttings. Hort. Abstr. 36, 515.

FLOOR, J., WEZELENBURG, P.A. & VON DOESBURG, H.H., 1956. Proeven met vermeerdering van houtagtige gewassen. Inst. Vered. Tuinbougewas. Wag. Mededeling 82.

GALSTRON, A.W. & PURVES, W.K., 1960. The mechanism of action of auxin. Ann. Rev. Plant Physiol. 11, 239 - 267.

GLADWIN, F.E., 1924. The grafting of american grapes. New York State Agric. Exp. Sta. N.Y. Bulletin No. 520.

HABERLANDT, G., 1921. Wundhormone als Erreger von Zellteilung. Beil. Allg. Bot. 2, 1 - 54.

HANSEN, C.J., 1964. Production and propagation of rootstocks.. Proc. Int. Plant. Prop. Soc. 14, 325 - 326.

HANSEN, C.J. & EGGERS, E.R., 1941. Propagation of fruit plants. Cal. Agric. Ext. Serv. Circular No. 96.

HANSEN, C.J. & HARTMANN, T., 1968. The use of Indolebutyric acid and Captan in the propagation of clonal peach and peach-almond hybrid rootstocks by hardwood cuttings. Proc. Amer. Soc. Hort. Sci. 92, 135 - 140.

HARMON, F.N. & SNYDER, E., 1948. Some factors affecting the success of green wood grafting of grapes. Proc. Amer. Soc. Hort. Sci. 52, 294 - 298.

HARMON, F.N. & WEINBERGER, J.H., 1967. Studies to improve the bench grafting of Vinifera grapes. Proc. Amer. Soc. Hort. Sci. 90, 149 - 152.

HARTMANN, H.T. & KESTER, D.E., 1968. Plant Propagation. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

HEYMANN-HERSCHBERG, L., 1949. The effect of synthetic growth substances on the rooting of grape vine cuttings. Palest. Jour. Bot. 7, 113 - 123.

HOWARD, G.S. & HILDRETH, A.C., 1963. Induction of callus tissue on apple grafts prior to field planting and its growth effects. Proc. Amer. Soc. Hort. Sci. 82, 11 - 15.

JACOB, H.E., 1942. Examples of incompatibility between grape varieties and rootstocks. Proc. Amer. Soc. Hort. Sci. 41, 201 - 203.

JAKES, E. & HEXNEROVA, H., 1939. Über den Einfluss der Wuchsstoffe auf Wundgewebebildung bei Obstbäumen. Gartenbauwiss. 13, 83 - 93.

JULLIARD, B., 1965. The effect of IAA on the callus and rooting of cuttings of Vitis berlandieri. Hort. Abstr. 35, 5304.

KLEIN, R.M. & VOGEL, H.H., 1956. Necessity of Indoleacetic acid for the duplication of crown-gall tumor cells. Plant Physiol. 31, 17 - 21.

KOCH, F. & LEIBIG, H., 1971. Wirkung verschiedener 8 - Hydroxychinolinsalze bei der Rebenveredlung. Weinberg und Keller 18, 451 - 464.

KORDES, H., 1938. Bedeutung der Wuchsstoffe für die vegetative Vermehrung der Rebe, insbesondere für die Rebenveredlung. Gartenbauwiss. 11, 545 - 554.

KRAUS, V., 1966. Rebenveredlung und Pfropfrebenbau in der Tschechoslowakei. Weinberg und Keller 13, 255 - 262.

LAIBACH, F., 1935. Über die Auslösung von Kallus- und Wurzelbildung durch β- Indolylessigsäure. Ber. Bot. Ges. 53, 359 - 364.

LEOPOLD, A.C., 1964. Plant Growth and Development. McGraw-Hill, Inc., U.S.A.

LIDER, L.A., 1954. Inheritance of resistance to a rootknot nematode (Meloidogyne incognita var. acrita (Litwood) in vitis spp. Proc. Helm. Soc. Washington 21, 53 - 60.

LIDER, L.A., 1960. Vineyard trials in California with nematode-resistant grape rootstocks. Hilgardia 30, 123 - 152.

LUCENKO, V.I. & LENINA, V.I., 1965. Paraffin treatment of grafts. Hort. Abstr. 35, 7378.

MALENIN, I., 1970. A trial on the effect produced by certain preparations on Agrobacterium tumefaciens and on the tumors of vines infected by bacterial cancers. Hort. Abstr. 40, 3446.

MISHURENKO, A.G. & NAGORNYI, N.I., 1962. Paraffining of vine grafts reduces the cost of transplants. Hort. Abstr. 32, 6228.

MISHURENKO, A.G. & SHTERENBERG, P.M., 1973. Application of Chinosol in the nursery. Hort. Abstr. 43, 3568.

NAKANO, H., 1924. Untersuchungen über Kallusbildung und Wundheilung bei Keimpflanzen II. Ber. deut. botan. Ges. 42, 267 - 272.

NESHEV, K. & VANEV. S., 1972. Testing Chinosol and Riedel paraffin for the control of grey rot on grafted cuttings. Hort. Abstr. 42, 811.

ORFFER, C.J., 1954. Wingerdoorentingstudies. Ongepubliseerde Tesis, Univ. Stellenbosch.

ORFFER, C.J., 1956. Ontogenetic and experimental studies on the establishment of the graft union in the grapevine. Ongepubliseerde Tesis, Univ. California.

ORFFER, C.J., 1957. Noodsaaklikheid van onderstoknavorsing vir die S.A. wynbou. Die Wynboer 312, 31 - 35.

ORFFER, C.J., 1966. Suksesvolle uitkenning van onderstokke. Pamflet Dept. Wingerdbou, S.E.L.K., Univ. Stellenbosch.

PEARSE, H.L., 1948. Die wortelvorming van wynstok- en pruimsteggies soos beïnvloed deur die voeding van die ouerplant en behandeling met planthormone. Dept. van Landbou, S.A. Wetenskaplike Pamflet No. 249.

PONGRáCZ, D.P., 1969. Lugenting met winterhout op groen lote. Die Wynboer 455, 29 - 31.

SCHENK, W., 1966. Kann die Ausbeute an pflanzfähigen Pfropfreben gesteigert werden? Weinberg und Keller 13, 53 - 64.

SCHENK, W., 1967. Untersuchen zur Anwendung von Wuchsstoffen in der Rebenveredlung. Wein-Wissenschaft 22, 1 - 16.

SCHENK, W., 1969. Stimulation der Veredlungspartner als Massnahme zur Erhöhung der Ausbeute an pflanzfähigen Pfropfreben. Weinberg und Keller 16, 13 - 30.

SCHRUFT, G., 1970. Die Wirkung des Botrytis-Präparates Benomyl im Amaranthus-Cytokinin-Test. Wein-Wissenschaft 25, 329 - 333.

SCHULZE, K., 1966. Veredlungswachse mit pilzhemmender Wirkung bei der Pfropfreben-herstellung. Weinberg und Keller 13, 37 - 47.

SHIPPY, W.B., 1930. Influence of environment on the callusing of apple cuttings and grafts. Amer. Jour. Bot. 17, 290 - 327.

SIEGEL, S., 1956. Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill Book. Co., INC., New York.

SIEVERS, E., 1971. Neue Wege bei der Versorgung der Rebenveredlung mit Edelreismaterial. Weinberg und Keller 18, 253 - 278.

SKENE, K.G.M., 1972. Cytokinin-like properties of the systemic fungicide Benomyl. Jour. hort. Sci. 47, 179 - 182.

SKOOG, F. & TSUI, C., 1948. Chemical control of growth and bud formation in tobacco stem segments and callus cultured in vitro. Amer. Jour. Bot. 35, 782 - 786.

SNOW, R., 1933. The nature of cambial stimulus. New. Phytol. 32, 288 - 296.

SPIEGEL - ROY, P., 1968. The effect of cold and hot treatments on the rooting percentage and quality of rooting of bench grafted grapevines. Hort. Abstr. 39, 4381.

STAHL, J.L., 1925. Propagation of deciduous fruits. Univ. of Cal. Circular No. 294.

STEPANOVA, A.F., 1970. Effect of growth stimulants and vitamins on root and callus formation in vine grafts. Hort. Abstr. 40, 5872.

SWINGLE, C.F., 1929. A physiological study of rooting and callusing in apple and willow. Jour. Agric. Res. 39, 81 - 127.

TIZIO, R., 1963. Effect of indolebutyric acid and biotin on the rooting and callusing of vine cuttings. Hort. Abstr. 33, 4753.

VAN DOESBURG, J., 1962. Use of fungicides with vegetative propagation. Proc. 16th Int. Hort. Cong. 4, 365 - 372.

VAN OVERBEECK, J., 1966. Plant hormones and regulators. Science 152, 721 - 731.

VOGT, E. & BRUNNER, E., 1949. Die Herstellung und Anpflanzung von Pfropfreben. Verlag für Landwirtschaft, Gartenbau und Naturwissenschaften, Stuttgart.

WELLS, J.S., 1963. The use of captan in rooting rhododendrons. Proc. Int. Plant. Prop. Soc. 13, 132 - 135.

WINKLER, A.J., 1927. Some factors influencing the rooting of vine cuttings. Hilgardia 2, 329 - 349.

WINKLER, A.J., 1962. General Viticulture. Univ. of Cal. Press, Berkeley and Los Angeles.